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Supercritical Fluid Chromatography for
High Molecular Weight Organic Analysis

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SUPERCRITICAL FLUID CHROMATOGRAPHY FOR HIGH MOLECULAR WEIGHT
ORGANIC ANALYSIS

by

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ABSTRACT

This report describes a preliminary application of supercritical fluid mass spectrometry techniques developed at Battelle-Northwest to the analysis of a middle distillate fuel, an emission particulate extract, and the emission particulates themselves. These techniques include capillary column supercritical fluid chromatography (alone) and coupled with mass spectrometry, direct fluid injection-mass spectrometry, and supercritical fluid extraction mass spectrometry. While these techniques are still in an early stage of development, they have demonstrated considerable potential for difficult analytical problems. The present work demonstrates the feasibility of achieving high resolution separations of complex mixtures with supercritical fluid chromatography using nonpolar carbon dioxide as the mobile phase. Additionally, these analyses have provided an evaluation of the limitations of the present detector interfaces and have resulted in further design improvements and emphasized research areas for further investigation. The present work also demonstrates that supercritical fluid chromatography-mass spectrometry application to complex mixtures can provide good separations and usable mass spectra. Direct fluid injection-mass spectrometry using supercritical ammonia was used to provide an efficient method of transporting sample components to the ionization source to obtain accurate molecular weight distribution data and structural information for rapid analysis of materials for which suitable separations have not yet been developed. Finally, high molecular species up to mass 1400 amu were detected during supercritical fluid extraction-mass spectrometry of the emission particulates using supercritical ammonia. The necessary research required to elevate the various supercritical fluid methods to routine application is summarized.

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I. INTRODUCTION

Recently, the interest and use of supercritical fluid (SF) phenomena in analytical applications has increased. A number of potentially important techniques have emerged which include capillary column supercritical fluid chromatography (SFC)(1-3), capillary column supercritical fluid chromatography-mass spectrometry (SFC-MS)(4,5), direct fluid injection (DFI)-mass spectrometry (MS)(6), and direct supercritical fluid extraction-mass spectrometry (SFE-MS)(7,8). While these techniques are in a relatively early stage of development, they have already shown considerable potential for providing important contributions to the characterization of complex mixtures. In this report the results of an initial application of these supercritical fluid techniques to the characterization of a middle distillate fuel, emission particulates, and the emission extract are described.

A supercritical fluid is a substance at a temperature above its critical temperature and pressure where a distinct liquid phase cannot exist. At elevated pressure this single phase will have properties intermediate between those of the gas and liquid phases and dependent upon the fluid composition, temperature and pressure. The compressibility of supercritical gases is large just above the critical temperature where small changes in pressure result in large changes in the density of the supercritical fluid(9). The density of a supercritical fluid will be typically 102 to 103 times greater than that of the gas at somewhat lower pressures. Consequently, molecular interactions increase due to the shorter intermolecular distances. The "liquid-like" behavior of a supercritical fluid results in greatly enhanced solubilizing capabilities compared to the "subcritical" gas, with higher diffusion coefficients and an extended temperature range compared to liquids. Compounds of high molecular weight can often be dissolved in the supercritical phases at low temperatures and the solubility of species up to 400,000 molecular weight has been demonstrated(10,11).

An interesting phenomenon associated with supercritical fluids is the occurrence of a "threshold pressure" for solubility of a high molecular weight solute(10). As the pressure is increased, the solubility of the solute will often increase by many orders of magnitude with only a small pressure increase(12). Thus, the threshold pressure is the pressure (for a given temperature) at which the solubility of a compound greatly increases (becomes detectable). This is the basis for the technique of pressure programming in supercritical fluid chromatography and the success of the selective SF extraction methods.

The analysis of complex mixtures is hindered by the molecular weight range and selectivity of present analytical instrumentation. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) are ultimately limited by the volatility of the particular chemical species to the study of compounds having molecular weight less than approximately 300 to 600. The use of supercritical fluid chromatography (SFC) offers a potential solution of these problems. The origin of SFC goes back nearly two decades, with a period of intense activity in the late sixties (10,11,13-20). The lack of sufficiently selective and sensitive detectors coupled with the rapid growth of high performance liquid chromatography (HPLC) led to a period where relatively little SFC progress was reported.

Liquid chromatography can be used to separate high molecular weight and less volatile compounds. However, the chromatographic resolution of HPLC is much less than with modern capillary column GC. The solvating capability of the mobile phase is often insufficient for large compounds containing several functional groups, and a sensitive and selective detector is required. Modern HPLC is rapidly approaching the practical limits on the number of effective theoretical plates and only minor improvements in chromatographic efficiency can be expected without new breakthroughs in column and pump technology. While some advances seem likely using packed capillary columns, open tubular columns for HPLC

suffer from the extremely small diameter required ($< 10 \text{ } \mu\text{m}$) for improvements in efficiency and associated difficulties related to sample size limitations, stationary phase preparation, and detection limits.

The ideal detector for HPLC is the mass spectrometer due to its inherent sensitivity and selectivity. However, interfacing these instruments is a difficult task and is limited due to fundamental incompatibilities in liquid flow rate into the mass spectrometer and solvent evaporation or removal. Existing HPLC-MS combinations to date have all proved to be lacking due to one or more difficulties which include: complexity, inadequate sensitivity, large variations in relative sensitivity, mass spectra complicated by pyrolysis or heterogeneous processes, inadequate (or degradation of) chromatographic resolution or poor interface operation. Thus, there is a clear need for an alternative approach to HPLC for analysis of complex mixtures which provides increased chromatographic resolution and greater compatibility with mass spectrometry.

The potential advantages of SFC accrue from the nature of the supercritical fluid itself(21). Table 1 compares the approximate magnitudes of important physicochemical properties of the supercritical fluid phase with liquid and gas phases. The lower viscosities and higher diffusion coefficients relative to liquids result in the potential for significantly enhanced chromatographic efficiency relative to liquid chromatography.

In SFC the mobile phase is maintained at a temperature somewhat above its critical point. The density of the supercritical phase is typically several hundred times greater than that of the gas, but significantly less than that of the liquid. The shorter intermolecular distances result in increased molecular interactions manifested by an increase in the solubilizing power of the mobile phase for the solute. This results in the capability of a much wider range of applications than available

TABLE 1
COMPARISON OF PROPERTIES OF GASES,
LIQUIDS AND SUPERCRITICAL FLUIDS

	Gas	Supercritical Fluid	Liquid
Density (g/cm ³)	10 ⁻³	0.3	1
Diffusion coefficient (cm ² /g)	10 ⁻¹	10 ⁻³ -10 ⁻⁴	<10 ⁻⁵
Viscosity (g/cm s)	10 ⁻⁴	10 ⁻⁴ -10 ⁻³	10 ⁻²

to GC, including the chromatography of very high molecular weight compounds (> 3000). The mild thermal conditions (determined by the choice of SFC mobile phase) allow the application to thermally labile compounds. Additionally, since the solvating capability of the mobile phase is dependent upon pressure, one has the capability for accurate control of this parameter. Thus in SFC, one can use pressure programming in a manner analogous to temperature programming in GC, while still having the potential for gradient or temperature programming. Another advantage of SFC is the substantially lower partition ratios observed relative to GC, resulting in the ability to chromatograph large molecules with reasonable retention times(15). Additionally, the use of open tubular capillary columns greatly reduces the pressure drop across the column, an important consideration if the pressure programming capability of SFC is to be exploited. Low pressure drops also allow relatively long column lengths to be utilized. Furthermore, open tubular columns offer extremely high

efficiencies with small diameter 50 μ m columns displaying over 3,000 effective plates/meter at K' values greater than 4.

The combination of SFC with mass spectrometry offers the following demonstrated(4,7) or potential advantages relative to both GC-MS and LC-MS methods:

- 1) High molecular weight, polymeric, heterofunctional and thermally labile compounds may be separated as well as volatile species(7).
- 2) Capillary open tubular SFC columns can provide greatly enhanced chromatographic resolution relative to HPLC due to diffusivities 10^1 to 10^2 greater than the liquid phase and viscosities similar to the gas phase(3,21).
- 3) Solvating power of the mobile phase can be readily controlled using pressure programming(1). Mixed mobile phases and temperature gradient programming are also feasible(21).
- 4) SFC using capillary columns allows low mobile phase flow rates which, coupled with high mobile phase volatility, provides for an optimum interfacing of SFC and mass spectrometry(4,7).

Randall and Wahrhaftig have previously reported on the construction of a supercritical fluid (dense gas) chromatograph-mass spectrometer interface using conventional packed columns and supersonic molecular beam techniques(22-24). This approach, however, suffers from the lower chromatographic resolution relative to capillary column techniques, the complexity of four stages of differential pumping required by large mobile phase flow rates, and mass spectra complicated by cluster formation during cooling in the beam expansion(24). Gouw et al. have also presented results using direct introduction with electron impact ionization but

this approach is limited by compound volatility and poor sensitivity(25). The relatively slow pressure drop during fluid introduction in their approach allows loss of the less volatile component.

The use of capillary column chromatography can obviate these difficulties. The SFC-MS interface developed at Battelle-Northwest allows injection of the total effluent into a modified mass spectrometer CI source. Small diameter open tubular capillary columns have been developed that are stable to the rigors of supercritical fluids and provide mobile phase flow rates compatible with mass spectrometry (<80 μ L/min). Pressure programming provides variation in solvating power to allow the elution of a wide molecular weight range of solutes. The method also allows the addition of an alternate CI reagent.

While still in a relatively early phase of development these techniques have shown considerable potential and have already demonstrated separations superior to HPLC. Further development of this approach will result in greatly enhanced analytical methodologies for wide classes of compounds.

II. CONCLUSIONS

A preliminary investigation of supercritical fluid mass spectrometry techniques for the analysis of complex mixtures has shown important potential. Although these techniques are still in an early stage of development and require additional improvements and modifications, it is clear that they are capable of providing important analytical characterization information. The following major conclusions can be made as a result of this preliminary study:

- High resolution separations of complex mixtures of moderate molecular weight (e.g., middle distillate fuel) were efficiently carried out using supercritical fluid chromatography with carbon dioxide as a mobile phase and the present flame ionization detector interface.
- In order to elute high molecular weight nonvolatile materials it will be necessary to modify the existing flame ionization detector interface and/or use fluids which upon decompression still provide solvating ability.
- Supercritical fluid chromatography-mass spectrometry of the middle distillate fuel using carbon dioxide as the mobile phase provided moderate resolution of the components. Isobutane CI spectra were also obtained. Minor modifications of the DFI interface will upgrade the separation efficiency similar to that of SFC.
- SFC-MS of the emission extract was not successful. This can be attributed to the sample (not enough originally supplied and no assurance of the quality of the extract obtained from the emission particulates) and to the loss of heavy molecular weight material in the DFI interface. For successful elution of heavy molecular weight material, modifications of the interface and/or use of more polar fluids will be necessary.

- DFI-MS of the middle distillate fuel, the chemical class fractions of the fuel, and the emission extract successfully provided NH_3 CI spectra. Essentially all the material was transported to the mass spectrometer ionization source. Molecular weight distributions and some structural information are obtainable from the DFI spectra.
- Direct supercritical fluid extraction-mass spectrometry of the particulate sample with ammonia demonstrated that material up to mass 1400 amu (limit of mass range) was extractable. The extraction profile obtained as a function of pressure showed a marked increase in extraction efficiency when supercritical pressures were reached and a lower yield as the sample was exhausted. The mass spectra which were obtained are very complex and additional investigations are necessary for interpretation.

III. RECOMMENDATIONS

The present report has provided experimental results using three new analytical techniques based upon supercritical fluids and using mass spectrometry. These techniques are capillary column SFC-MS, direct fluid injection-MS, and supercritical fluid extraction-MS. While these techniques are all in a relatively early stage of development, they show significant promise for application to previous intractable analytical problems or for considerable simplification of established methodologies. The advantages of these methods include greatly enhanced chromatographic resolution compared to HPLC and improved operation with mass spectrometry. These techniques, which have been developed at Battelle-Northwest, require further developmental work to define their area of applicability, possible limitations, and to improve instrumentation technology. Research needs for continued development of this technology includes:

- Further development of columns for capillary SFC with appropriate stationary phases with polar fluids. This includes evaluation of gas preparation and purification procedures, gradient elution techniques for capillary SFC, and improved sample injection and flow control techniques.
- Exploration of polar fluid systems required for more polar solutes using DFI-MS.
- Coupling capillary SFC with high resolution MS to exploit the expanded analytical capability.
- Exploration of SF prefractionation with DFI-MS to reduce sample preparation and handling.

IV. EXPERIMENTAL

Samples

Samples were provided by EPA/AEERL and consisted of the following: (1) a middle distillate fuel sample (B103B), (2) emission particulates, MAV scrapings (B103C), and a (3) MAV extract (B103C).

Sample Preparation

An alumina adsorption chromatography scheme(26) was used to fractionate the fuel into discrete chemical classes to facilitate further analysis.

Neutral aluminum oxide (Brockman Activity 1, 80-200 mesh, Fisher No. A950) was standardized by appropriate heat treatments (calcination) and water addition. Approximately 0.2g of the fuel was dissolved in a few milliliters of chloroform and adsorbed onto 3g of the alumina. After evaporation of the chloroform the alumina was packed on top of an 11 mm i.d. column which was already packed with 6g of alumina. The sample was then eluted with the following high purity solvents: fraction A-1, 20 mL of hexane; fraction A-2, 50 mL of benzene; fraction A-3, 70 mL of chloroform containing 0.75% ethanol; and fraction A-4, 50 mL of 10% ethanol in tetrahydrofuran. Aliphatic hydrocarbons elute in the A-1 fraction, neutral polycyclic aromatic compounds including sulfur heterocycles elute in the A-2 fraction, nitrogen containing polycyclic aromatic compounds including carbazoles, aza-arenes, and aromatic amines elute in the A-3 fraction, and hydroxy aromatic compounds elute in the A-4 fraction.

SUPERCritical FLUID CHROMATOGRAPHY

The instrumentation used for supercritical fluid chromatography (SFC) is similar to that described elsewhere(3). The SFC apparatus

utilizes a modified Varian 8500 syringe pump to generate the high pressure pulse-free mobile phase supply and a modified HP 5700 GC oven to provide constant temperature conditions. Carbon dioxide was used for the supercritical fluid mobile phase, and since flame ionization detection was utilized it was absolutely imperative to purify and remove any hydrocarbon impurities from the liquid carbon dioxide. This was done by distilling the liquid into the pump while passing it through the appropriate adsorbents. Pressure operating conditions were controlled with a Hewlett-Packard 9845B desktop computer. A Valco C6W HPLC injection valve with a 0.2 μ l sample loop mounted exterior to the oven was used for sample introduction. The chromatographic column was mounted to the injector through a splitter(27). The restriction on the split vent was adjusted to allow approximately a 1:100 flow into the chromatographic column. The interface between the terminal end of the column and the flame ionization detector consisted of a short length of 4 μ m i.d. fused silica tubing. A 20m x 50 μ m i.d. fused silica capillary column coated with a 0.25 μ m film-thickness of a specially cross-linked 50% phenyl polymethylphenylsiloxane stationary phase was used for all analyses. The oven was maintained at 40°C and a pressure profile of 72 atm held isobarically for 20 minutes after injection followed by a 1 atm/min pressure ramp to approximately 150 atm was followed. The linear velocity of the supercritical mobile phase supercritical was adjusted to approximately 3 cm/sec.

Supercritical Fluid Chromatography-Mass Spectrometry

The instrumentation used for supercritical fluid chromatography-mass spectrometry (SFC-MS) has been described in detail elsewhere(4,5). A schematic diagram of this instrumentation is shown in Figure 1. Supercritical carbon dioxide was used for the chromatographic mobile phase and isobutane for the CI reagent gas. The high pressure pulse-free liquid flow rate was generated using a modified Varian 8500 syringe pump. Pressure programming was achieved with a microprocessor controller.

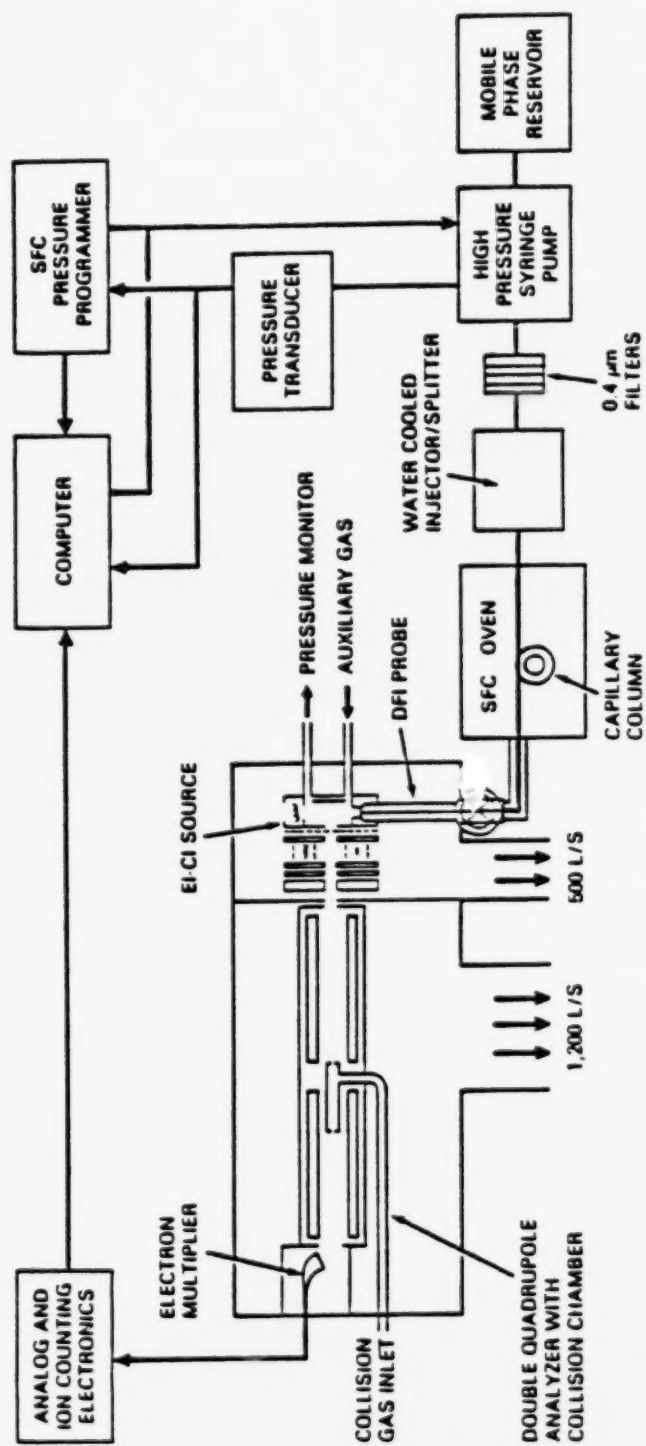


FIGURE 1. Schematic Illustration of the SFC-MS Instrumentation

Prior to introduction into the pump the carbon dioxide was cleaned using various filters and adsorbents. Sample introduction was accomplished using a 0.2 μ l Valco HPLC injector operated at ambient temperature. The chromatographic capillary column (50 μ m i.d) was connected to the injector via a sample splitter. The chromatographic column was maintained at a constant temperature of 40°C (slightly above the critical temperature) by placing it in a constant temperature bath which also provided circulation for the DFI probe. The column extended to within 3 cm of the DFI probe tip where it was connected to a short piece of platinum-iridium tubing which served as a connection to the DFI orifice. The linear velocity of the mobile phase was adjusted to approximately 3 cm/sec. The capillary column was similar to the one used for the SFC analyses. The injection port splitter was operated at a split ratio of approximately 100 to 1. Pressure programming rates of 1 atm/min from 72 atm to 150 atm or greater were used. The pressure was held isobarically at 72 atm until after elution of the solvent peak. A scan range of 90-400 amu was typically used.

Direct Fluid Injection-Mass Spectrometry

The instrumentation used for direct fluid injection-mass spectrometry (DFI-MS) is similar to that previously described for SFC-MS. A schematic illustration of the DFI-MS instrumentation is shown in Figure 2. Ammonia was used for the supercritical fluid (chosen because of its good solvating properties) and was distilled and cleaned using various filters and adsorbent materials prior to use. The required high pressure pulse-free liquid flow was generated using a Varian 8500 syringe pump (8000 p.s.i. maximum pressure). The liquid flow was filtered and cleaned before the temperature was elevated above the critical temperature. An 0.2 μ l Valco HPLC injector valve was operated at ambient temperature. The elevation to supercritical temperatures occurred rapidly after injection (<1 sec) to prevent loss of material due to possible limited solubility

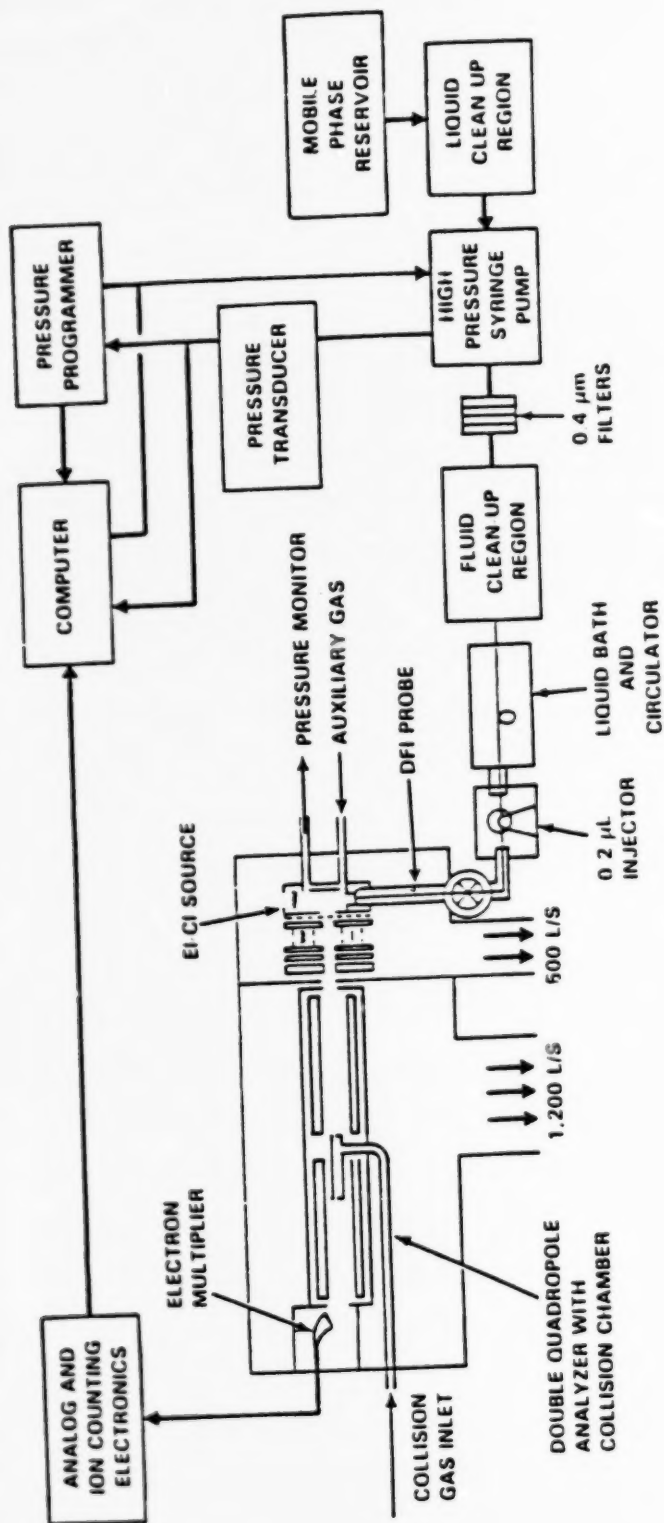


FIGURE 2. Schematic Illustration of the Direct Fluid Injection-Mass Spectrometer.

in the subcritical liquid. The injector was mounted at the end of the DFI probe which may be conveniently removed from the vacuum chamber using a standard 0.5 inch diameter direct insertion interlock. A length of approximately 35 cm of the 0.1 mm i.d. platinum-iridium tubing provided transfer between the injector and DFI orifice while maintaining a total volume of approximately 3 μ L. The DFI probe was heated by fluid circulation to maintain isothermal conditions up to the DFI orifice. Fluid circulation provides probe operation up to 200°C with temperature gradients less than 1°C. In general, exact and uniform control of temperature is unimportant when operating at sufficiently high pressures and temperatures where fluctuations of fluid solvating characteristics are minor. The flow rate is typically in the range of 2-30 μ L/min, and is determined by the DFI probe restrictor (orifice). Fluid flow rates are chosen to be typically 50% to 90% of the optimum CI source pressure. The remaining gas flow to the CI source is provided through an additional gas inlet. Ammonia was also used as the CI reagent in this work. Sample solvent composition and sample size are chosen so that supercritical conditions are maintained for the transfer line and DFI probe.

In the DFI-MS analysis subtle effects related to enhanced solubility due to the sample solvent may be observed for compounds of marginal solubility, depending upon the flow rate. For 0.2 μ L sample injections, the sample solvent may have a significant effect upon solubility due to the 3 μ L total volume available for mixing during transfer. The enhanced solvating power of the resultant fluid mixture can result in transfer and detection of otherwise insoluble compounds between the injector and DFI orifice. Thus, all sample injections are preceded and followed by blank solvent injections to remove any such material prior to analysis and to confirm complete transfer of material.

Supercritical Fluid Extraction - Mass Spectrometry

The instrumentation used for the supercritical fluid extraction-mass spectrometry (SFE-MS) is similar to that previously described for the DFI-MS analyses. A schematic illustration is shown in Figure 3. In these studies the emission particulates were packed into a small volume(50 uL) extraction cell and connected in-line with the DFI probe. Ammonia was used exclusively as the supercritical fluid solvent due to its excellent solvating properties. Naturally, ammonia was also used as the CI reagent gas. For these extractions, a mass range of 100 to 1400 amu was continuously scanned. The extraction cell and DFI probe were maintained at a constant temperature of 140°C (slightly above the critical temperature of ammonia). The pressure was then ramped in a linear fashion at a rate of 2.5 atm/min between 25 atm and 450 atm as mass spectra were continuously recorded. The relatively small extraction cell dead volume and the fluid flow rate of 20 ul/min were designed to allow changes in the extracted species with pressure to be readily observed. All analyses were preceded by "blank" experiments to give background spectra from impurities in the extraction cell and/or clustering effects from the CI process.

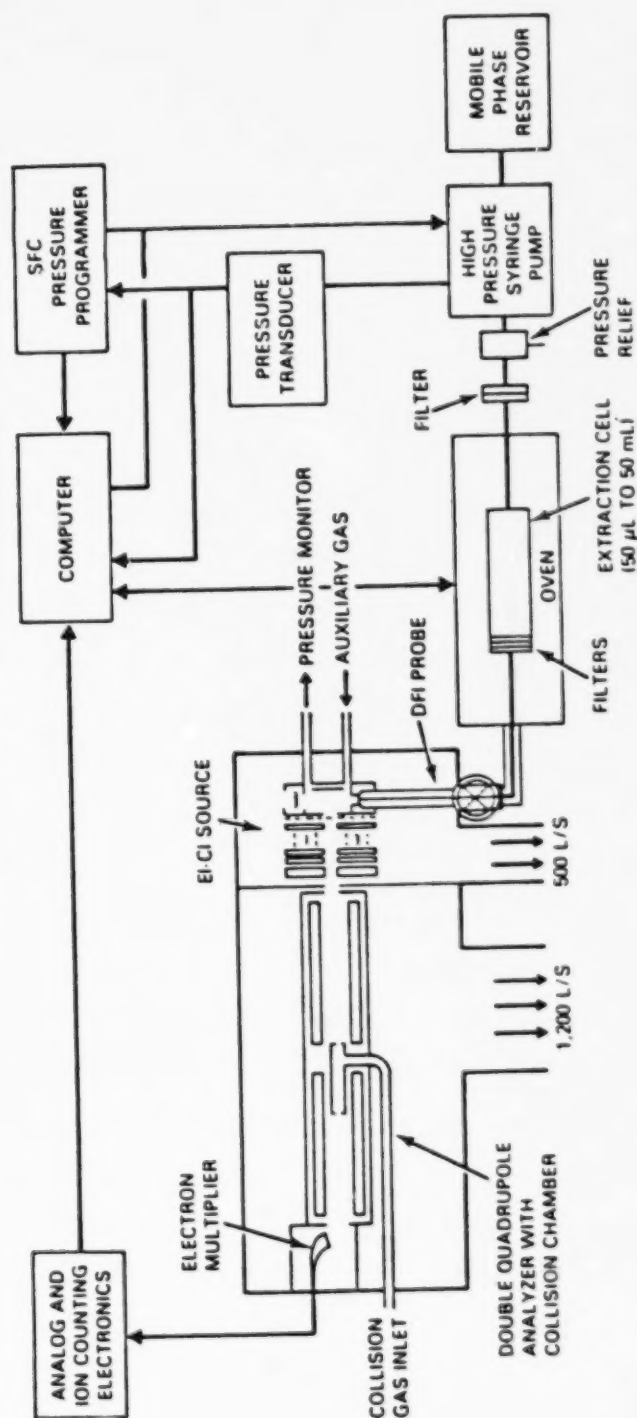


FIGURE 3. Schematic Illustration of the Supercritical Fluid Extraction-Mass Spectrometer

V. RESULTS AND DISCUSSION

Sample Fractionation

The weight distribution of the fractionated fuel is given in Table 2. The total recovery of the fuel obtained from three replicate separations was essentially quantitative (98%). The fuel was essentially aliphatic material (A-1) with some aromatic hydrocarbons (A-2 and hydroxylated material (A-4).

Table 2
WEIGHT DISTRIBUTION OF MIDDLE DISTILLATE FUEL

Fraction	Normalized Weight Percent
A-1 (aliphatic hydrocarbons)	70.8%
A-2 (neutral polycyclic aromatic hydrocarbons)	14.8%
A-3 (nitrogen-polycyclic aromatic hydrocarbons)	2.2%
A-4 (hydroxy-polycyclic aromatic hydrocarbons)	12.2%

Supercritical Fluid Chromatography

Carbon dioxide, a nonpolar fluid, was used for the SFC investigations due to its compatibility with flame ionization detection and its relative ease of use. Unfortunately, polar fluid systems have not yet been advanced to the point where reliable and reproducible operation are achievable. However, significant progress has been made and with continued effort adequate technologies will be developed.

The middle distillate fuel, the four fractions obtained from the fuel, and the emission extract were all chromatographed. Capillary supercritical fluid chromatograms are shown in Figures 4-9. The chromatogram of the total fuel sample is shown in Figure 4. It is evident that the fuel is a very complex mixture with a high concentration of minor components (evidenced from the "hump" formed from unresolved, yet eluted material). It also appears that the material consists of a moderate molecular weight range (discussed later). The chromatograms of the four chemical class fractions shown in Figure 5-8 also depict extremely complex mixtures. It is noteworthy that the more polar compounds (fractions A-3 and A-4 containing the nitrogen heterocycles and the hydroxylated and phenolic compounds, respectively) were also successfully chromatographed and eluted with supercritical carbon dioxide.

Chromatography of the emission extract was not as successful. The chromatogram obtained for this sample is shown in Figure 9. The shape of the chromatographic profile indicates that material was eluted, but not successfully resolved. Unfortunately, the quantity of original extract which was supplied was not sufficient for adequate experimentation. A small amount of additional extract was obtained by Soxhlet extraction with methylene chloride of the emission particulate sample (used for the analysis shown in Figure 9), but it is questionable if this extract was similar to the original extract. In any case, the instrumental configuration which was used does not appear to successfully chromatograph the nonvolatile portion of the sample. This is due to material-loss in the detector interface. During fluid decompression the nonvolatile material precipitates prior to reaching the detector. Several approaches are currently being implemented to overcome this problem.

In these chromatographic investigations, 20 meter column lengths were utilized, but columns up to at least 50 meters would be practical. Consequently, twice the efficiency could be easily achieved. Efficiency

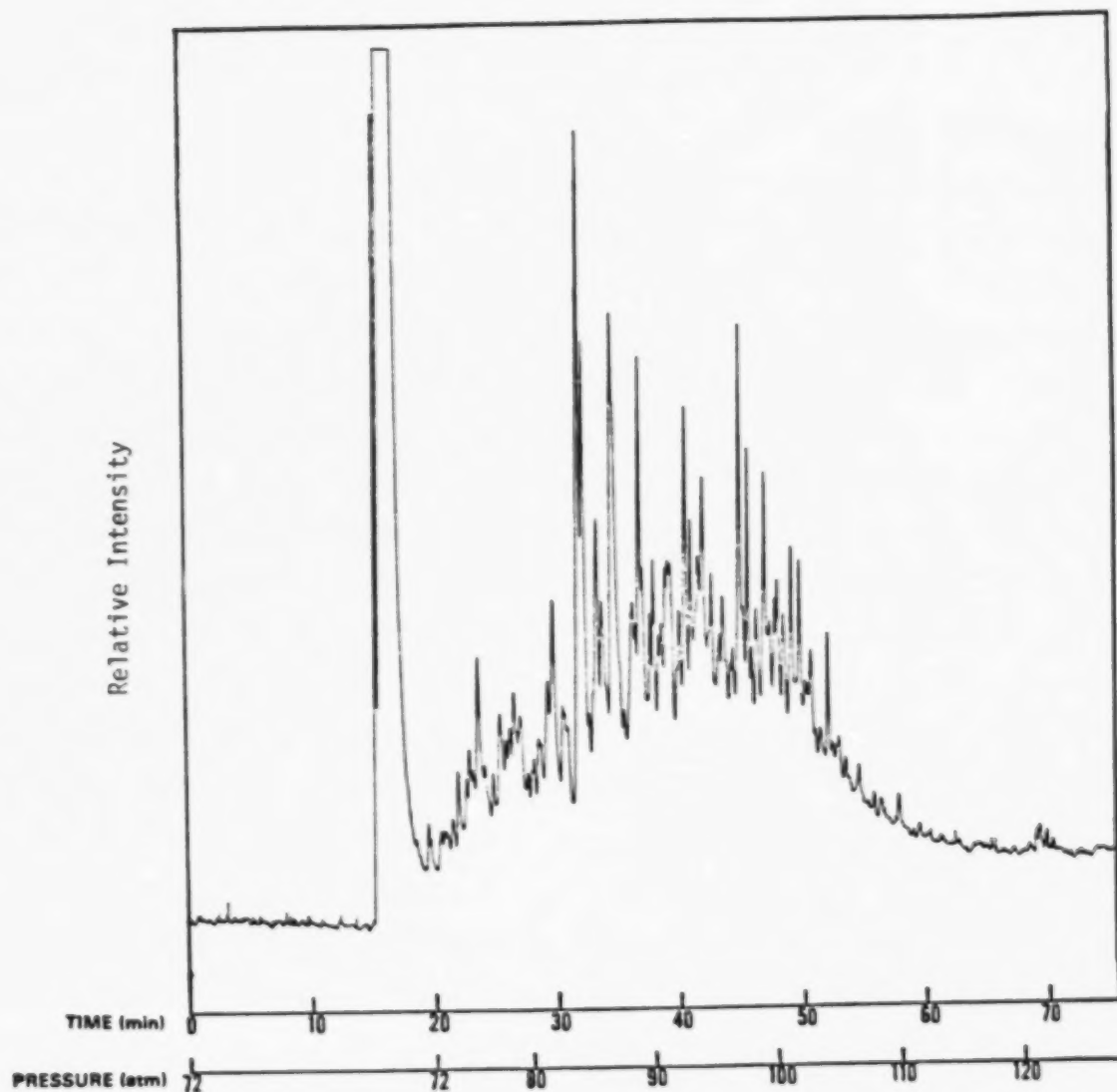


FIGURE 4. Capillary Supercritical Fluid Chromatogram of the Middle Distillate Fuel. Chromatographic Conditions: 20m x 50 μ m ID Fused Silica Column Coated with a 0.25 μ m Film Thickness of Crosslinked 50% Phenyl Polymethylphenylsiloxane Stationary Phase. CO_2 Fluid at 40°C and a Linear Velocity of About 3 cm/sec. Flame Ionization Detection. Pressure Ramped from 72 to 150 atm at 1 atm/min after 20 min at 72 atm Following Injection.

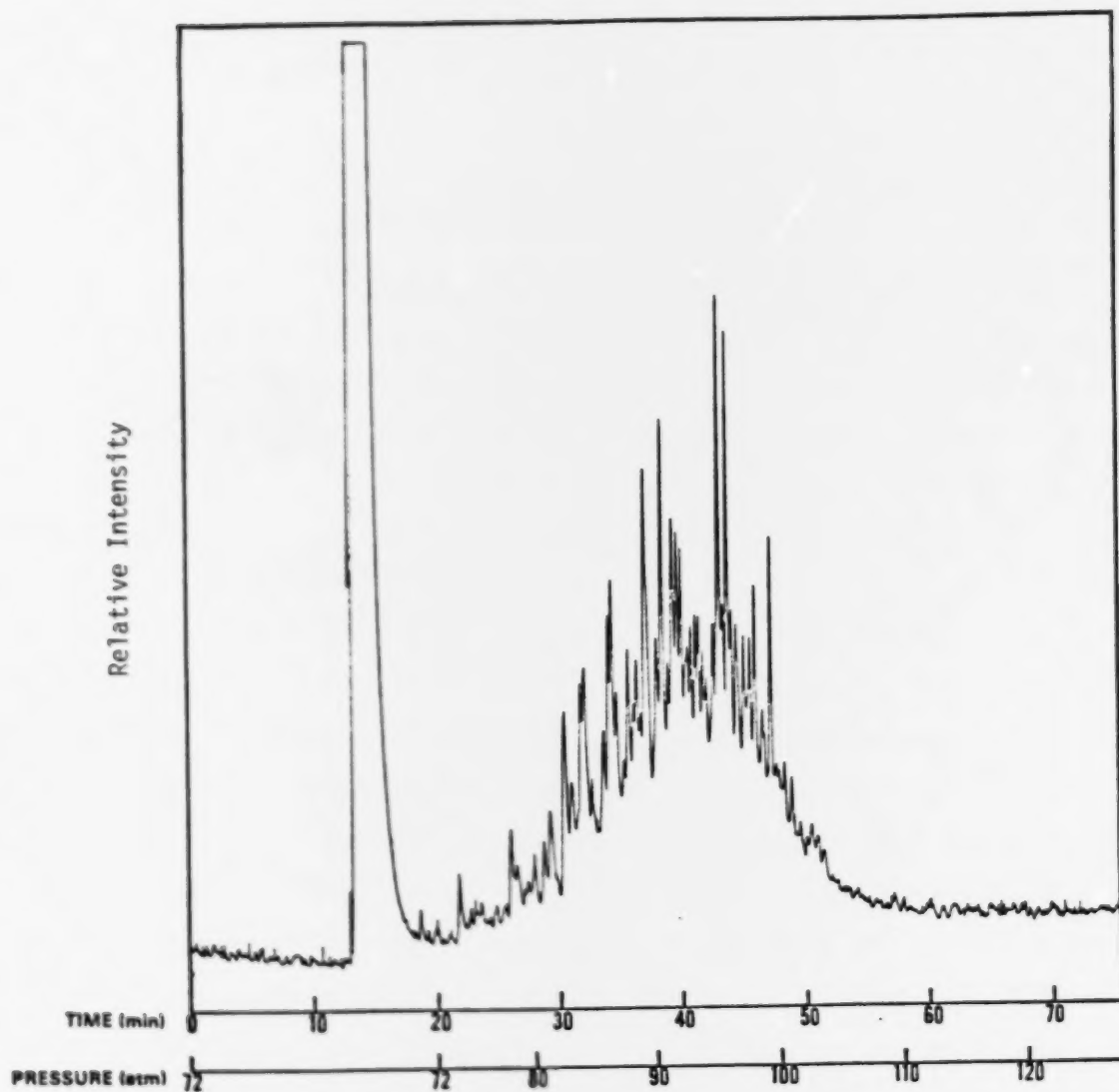


FIGURE 5. Capillary Supercritical Fluid Chromatogram of the Aliphatic Fraction of the Middle Distillate Fuel. See Figure 4 for Chromatographic Conditions.

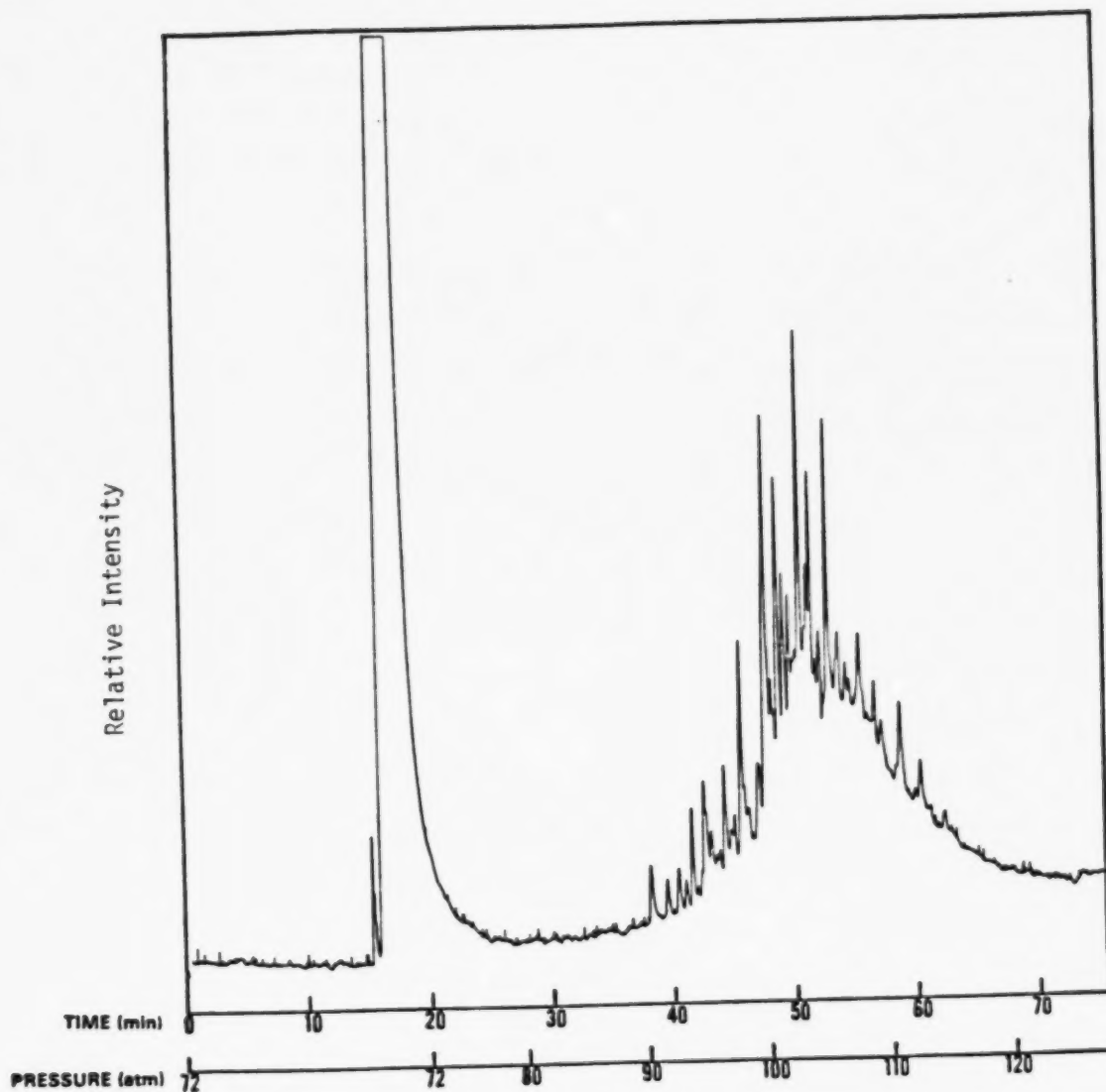


FIGURE 6. Capillary Supercritical Fluid Chromatogram of the Polycyclic Aromatic Hydrocarbon Fraction of the Middle Distillate Fuel. See Figure 4 for Chromatographic Conditions.

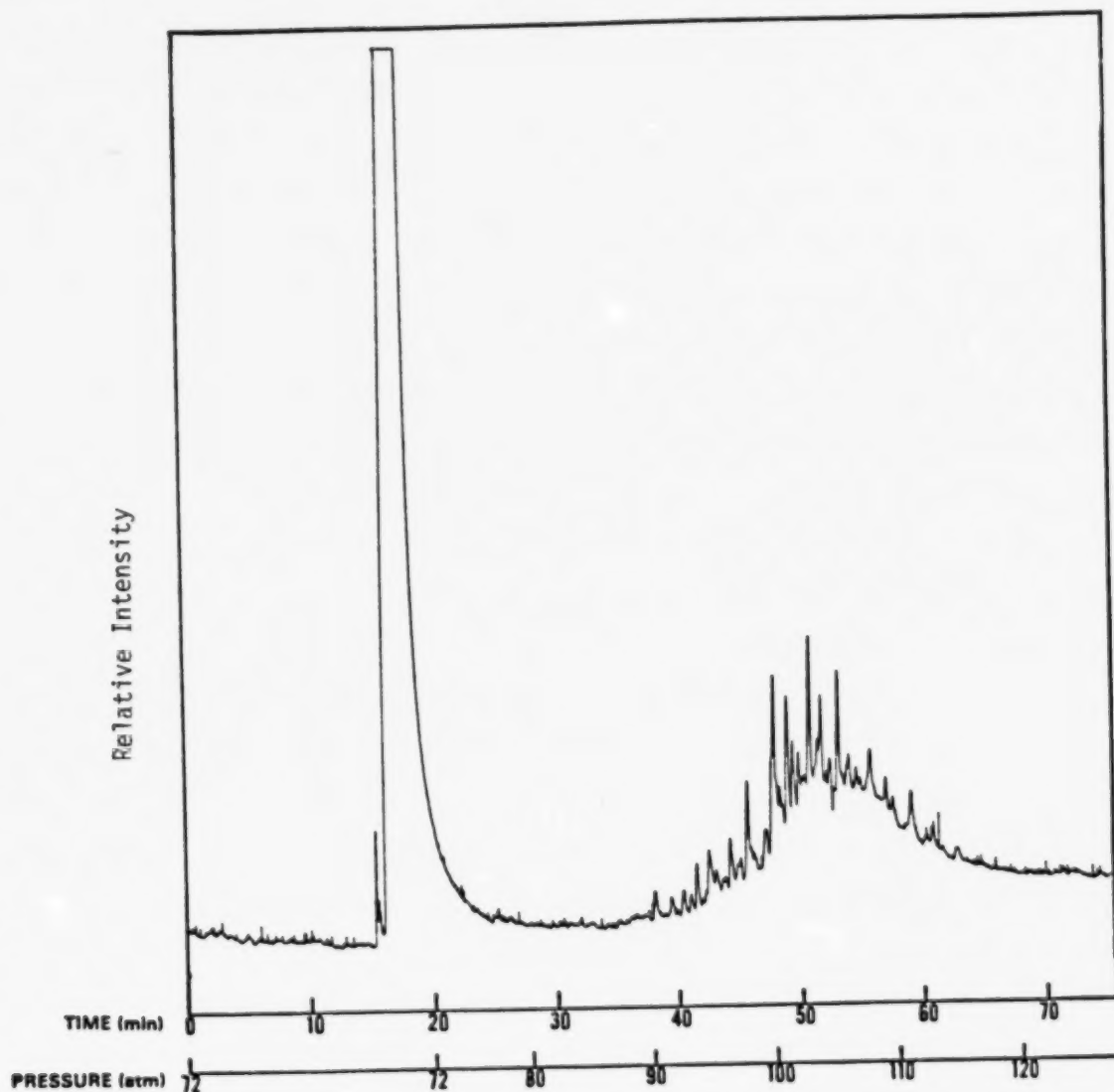


FIGURE 7. Capillary Supercritical Fluid Chromatogram of the Nitrogen-Containing Polycyclic Aromatic Hydrocarbon Fraction of the Middle Distillate Fuel. See Figure 4 for Chromatographic Conditions.

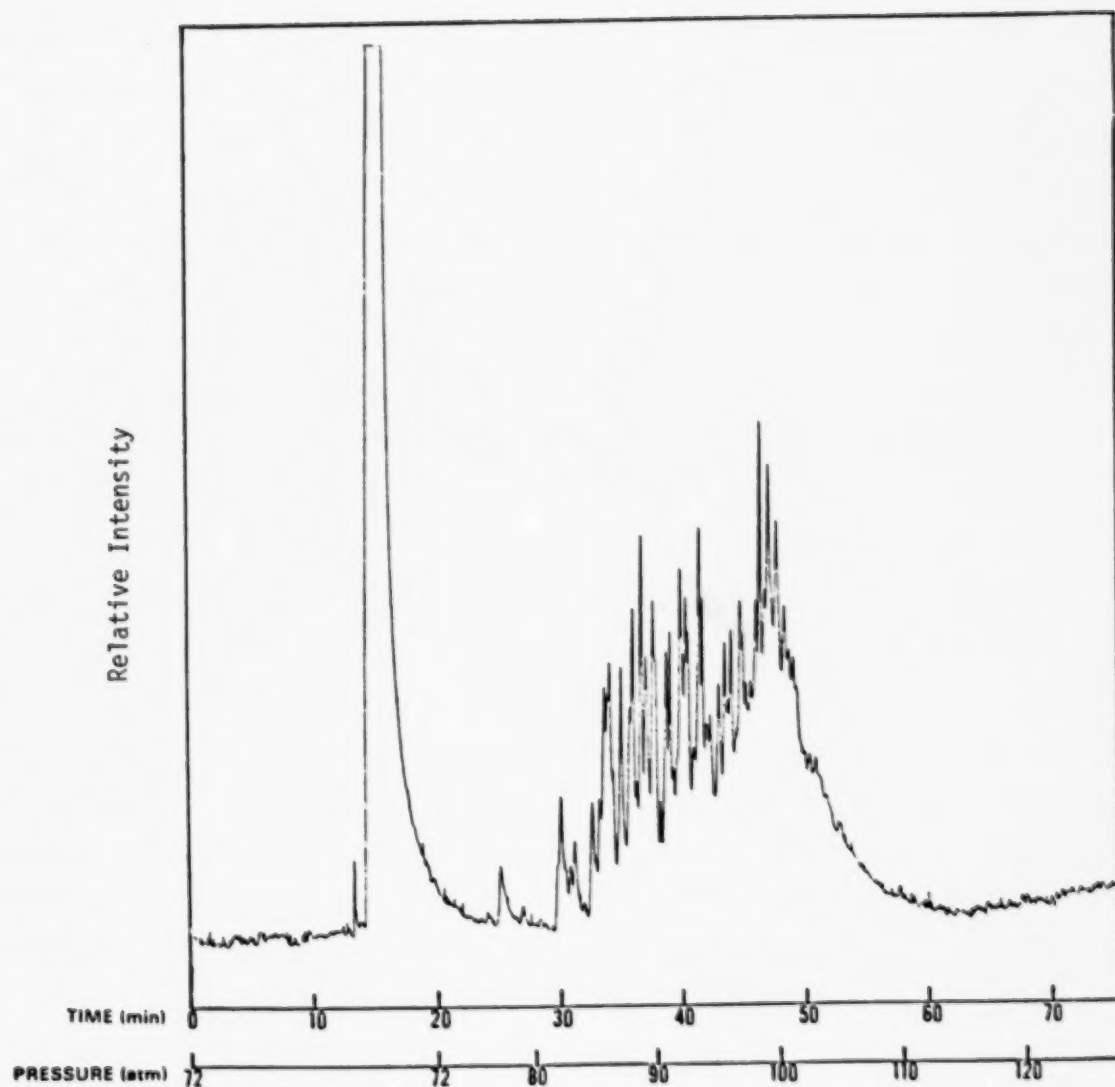


FIGURE 8. Capillary Supercritical Fluid Chromatogram of the Hydroxy Polycyclic Aromatic Hydrocarbon Fraction of the Middle Distillate Fuel. See Figure 4 for Chromatographic Conditions.

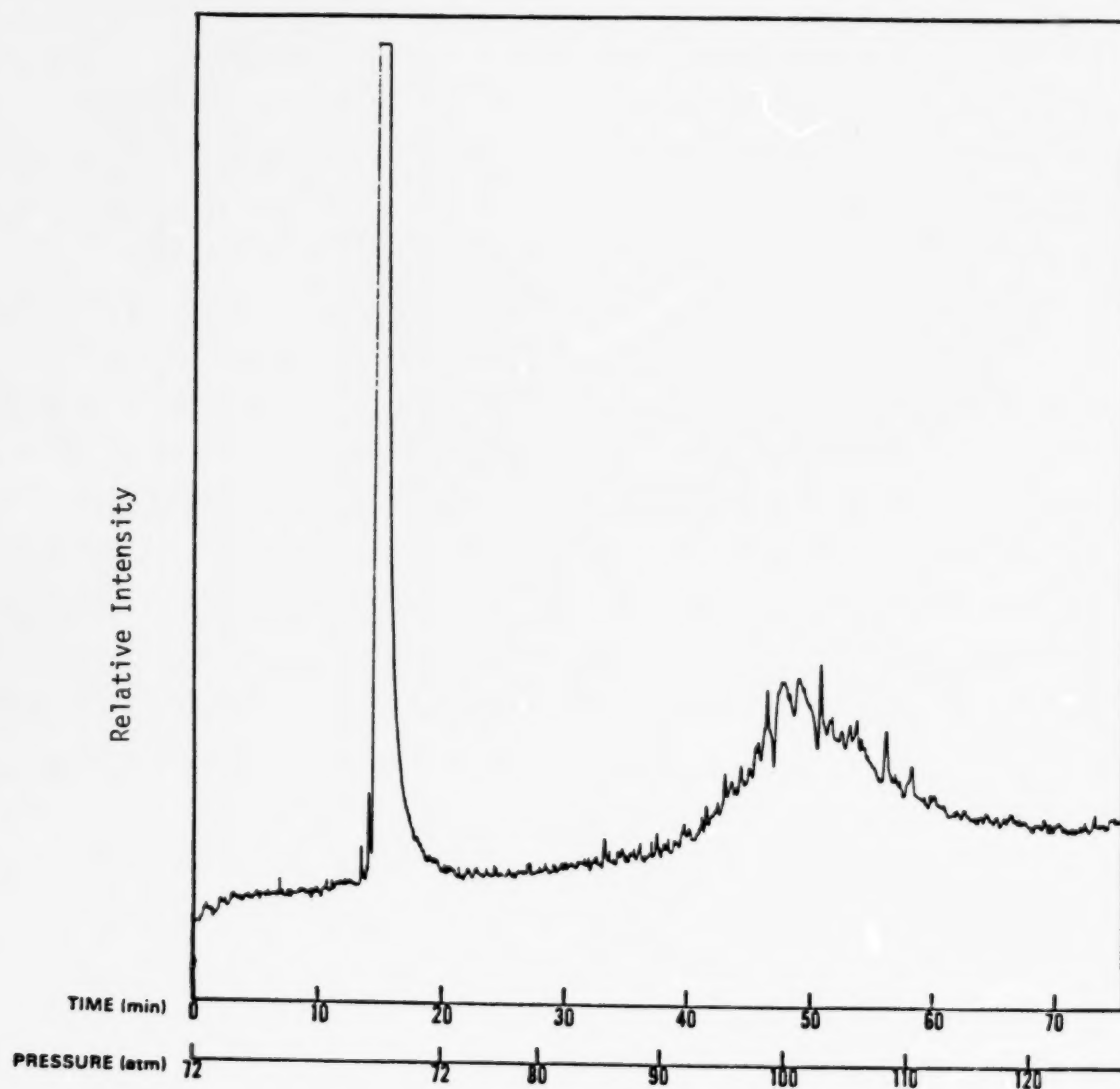


FIGURE 9. Capillary Supercritical Fluid Chromatogram of the Emission Extract. See Figure 4 for Chromatographic Conditions.

calculations indicate that about 3000 plates/meter can be generated for compounds with $K' > 3$ (more for compounds with lower K') using the same experimental conditions as were utilized in these experiments. Increased resolution of the sample components could also be obtained by using more precise pressure control such as linear density or asymptotic density profiles. It is evident from these preliminary evaluations that supercritical fluid chromatography has the potential of providing high resolution analyses of complex mixtures. However, due to theoretical reasons, the efficiency will never be as high as obtainable in gas chromatography. With appropriate detector interfacing and use of polar fluids, high molecular weight nonvolatile material could also be successfully eluted.

Supercritical Fluid Chromatography - Mass Spectrometry

The total-ion-plot obtained for an SFC-MS analysis of the middle distillate fuel is shown in Figure 10. Although the efficiency of this separation was less than that obtained by SFC alone, the potential is the same. Only minor improvements in the DFI interface will significantly decrease the band broadening and greatly improve the efficiency. A series of selected-ion-plots obtained from the same analysis are shown in Figure 11. The molecular weight range of the fuel appears to center around 150 m/Z with an upper limit of approximately 250 m/Z. Selected spectra obtained during chromatographic peaks are shown in Figure 12. Isobutane CI mass spectra given predominate (M+1), (M-1), and (M+57) ions.

SFC-MS analysis of the emission extract was not successful. The methylene chloride extract was also used for these trial analyses. Essentially, only very limited amounts of material were eluted. Again, the heavier molecular weight material was probably lost in the DFI interface. Decompressed (gaseous) supercritical carbon dioxide is not a good solvent and the nonvolatile material is lost. Use of more polar fluid systems in which the decompressed fluid is still a good solvent

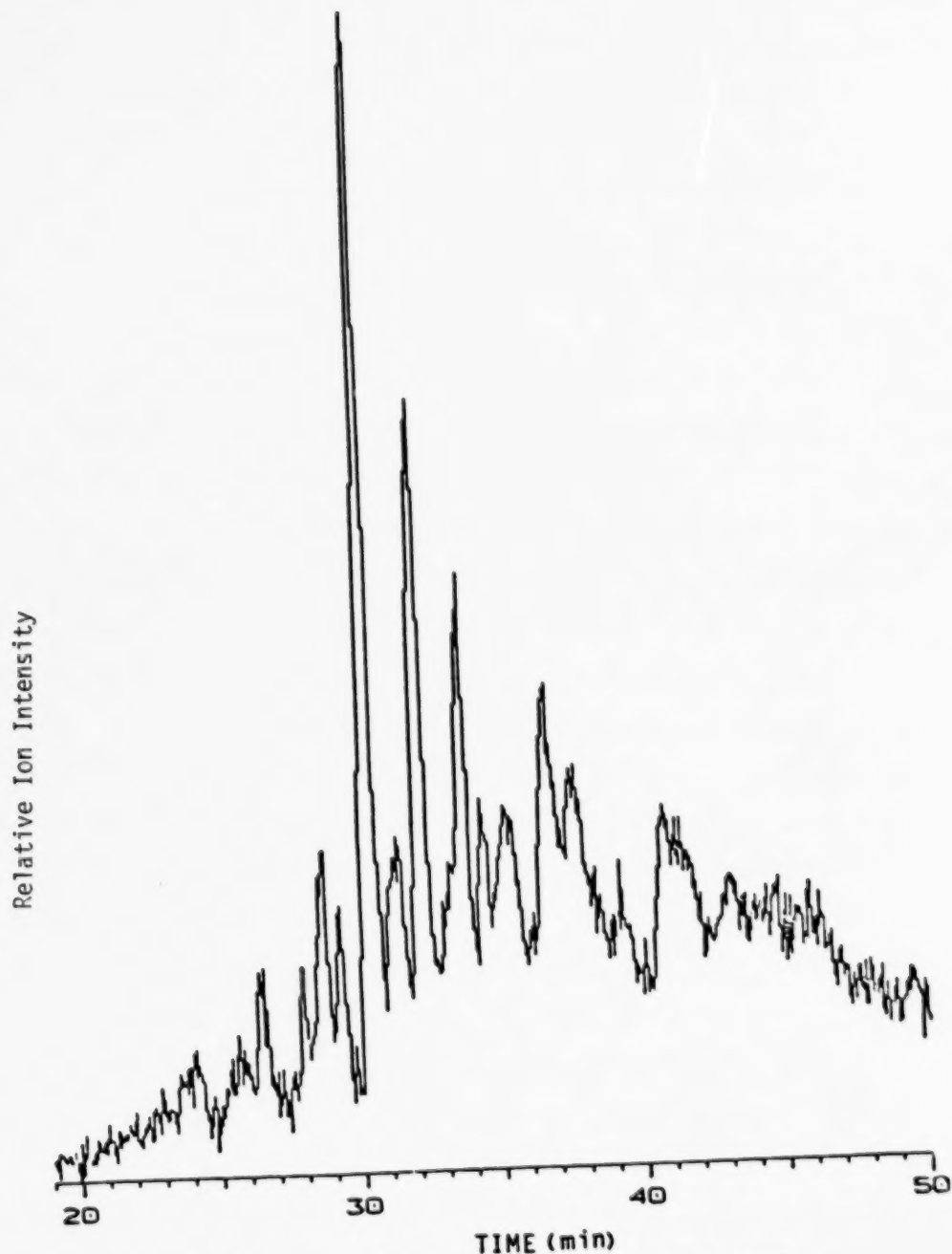


FIGURE 10. Total Ion Plot Obtained by SFC-MS of the Middle Distillate Fuel. Chromatographic Conditions: 20m x 50 μ m ID Fused Silica Column Coated with a 0.25 μ m Film-Thickness of Crosslinked 50% Phenyl Polymethyl Phenylsiloxane Stationary Phase. CO₂ Fluid at 40°C and a Linear Velocity of about 3 cm/sec. Pressure Ramped from 72 to 150 atm at 1 atm/min after 20 min at 72 atm Following Injection. Mass Spectrometer Scan Range of 100-400 amu.

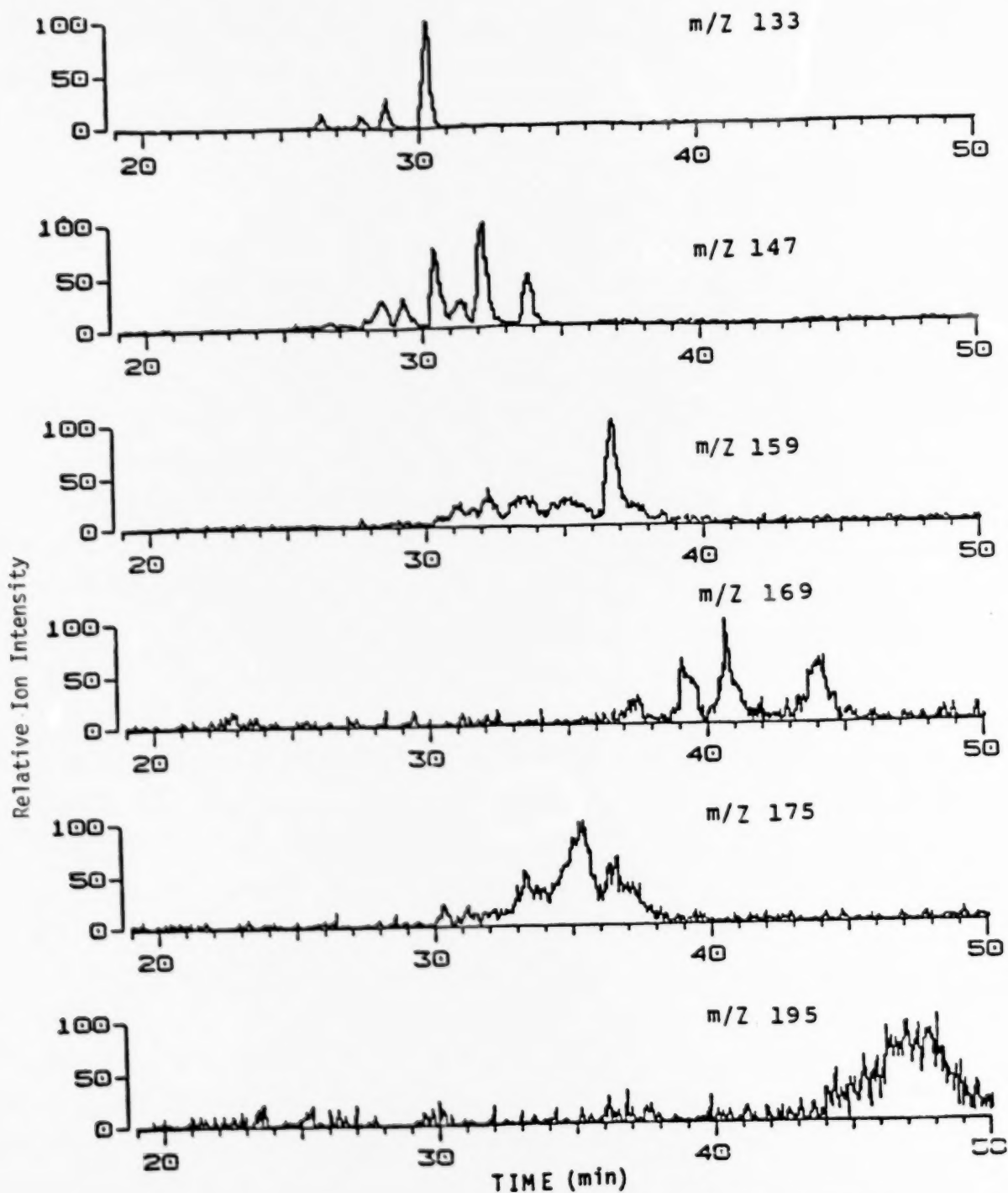


FIGURE 11. Selected Ion Plots Obtained During SFC-MS of the Middle Distillate Fuel. See Figure 10 for Conditions.

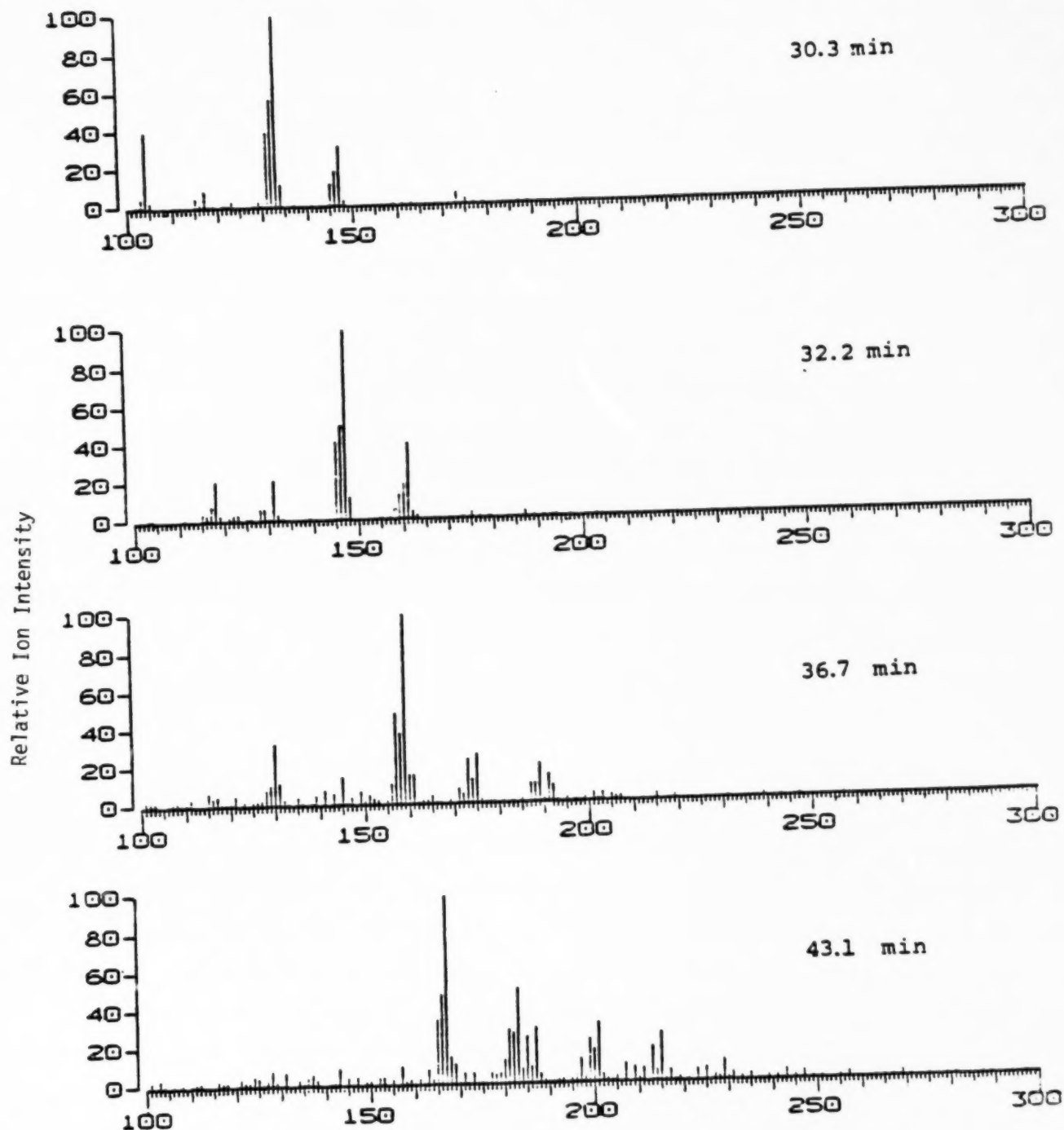


FIGURE 12. Mass Spectra at Selected Time Intervals Obtained During SFC-MS of the Middle Distillate Fuel. See Figure 10 for Conditions.

(e.g., pentane) would greatly extend the molecular weight range and warrants investigation.

Direct Fluid Injection - Mass Spectrometry

DFI-MS spectra were obtained for the middle distillate fuel, the four fractions of the fuel, and the emission extract. The DFI-MS method has the advantage of allowing a relatively rapid analysis. Assuming complete solubility in the supercritical ammonia, all the material was transported rapidly to the mass spectrometer ion source. This approach avoids the discrimination due to volatility inherent in other mass spectrometric inlet techniques. Supercritical ammonia was chosen for the supercritical fluid because of its excellent solvating properties. Previous experience has shown that even nonpolar materials are soluble in polar supercritical ammonia. Additionally, NH_3 CI provides some selectivity for polar (nonvolatile) components of high proton affinity. An example illustrating the utility of NH_3 DFI-MS is given in Figure 13 where the spectrum of 2-deoxyadenosine is shown.

The DFI-MS spectra obtained for the middle distillate fuel are shown in Figure 14. These spectra were obtained at 140°C and 450 atm using NH_3 as the CI reagent gas. The spectra define a molecular weight distribution ranging to nearly 350 amu, but gave no evidence (even at higher gain) of any heavier molecular weight material. Since only $(M+1)$ or $(M-1)$ ions predominate under the mild ionizing conditions obtained with DFI-MS, the complexity of the spectra certainly suggests that the sample is extremely complex. DFI-MS spectra for the chemical class fractions of the fuel are shown in Figures 15 to 18. Although much simpler, these spectra are also quite complex. The aliphatic hydrocarbon fraction shown in Figure 15 has a maximum molecular weight of approximately 254 corresponding to a C_{18} type hydrocarbon. A regular spacing in masses of 14 amu also suggests a homologous series of hydrocarbons. The higher molecular weight clusters

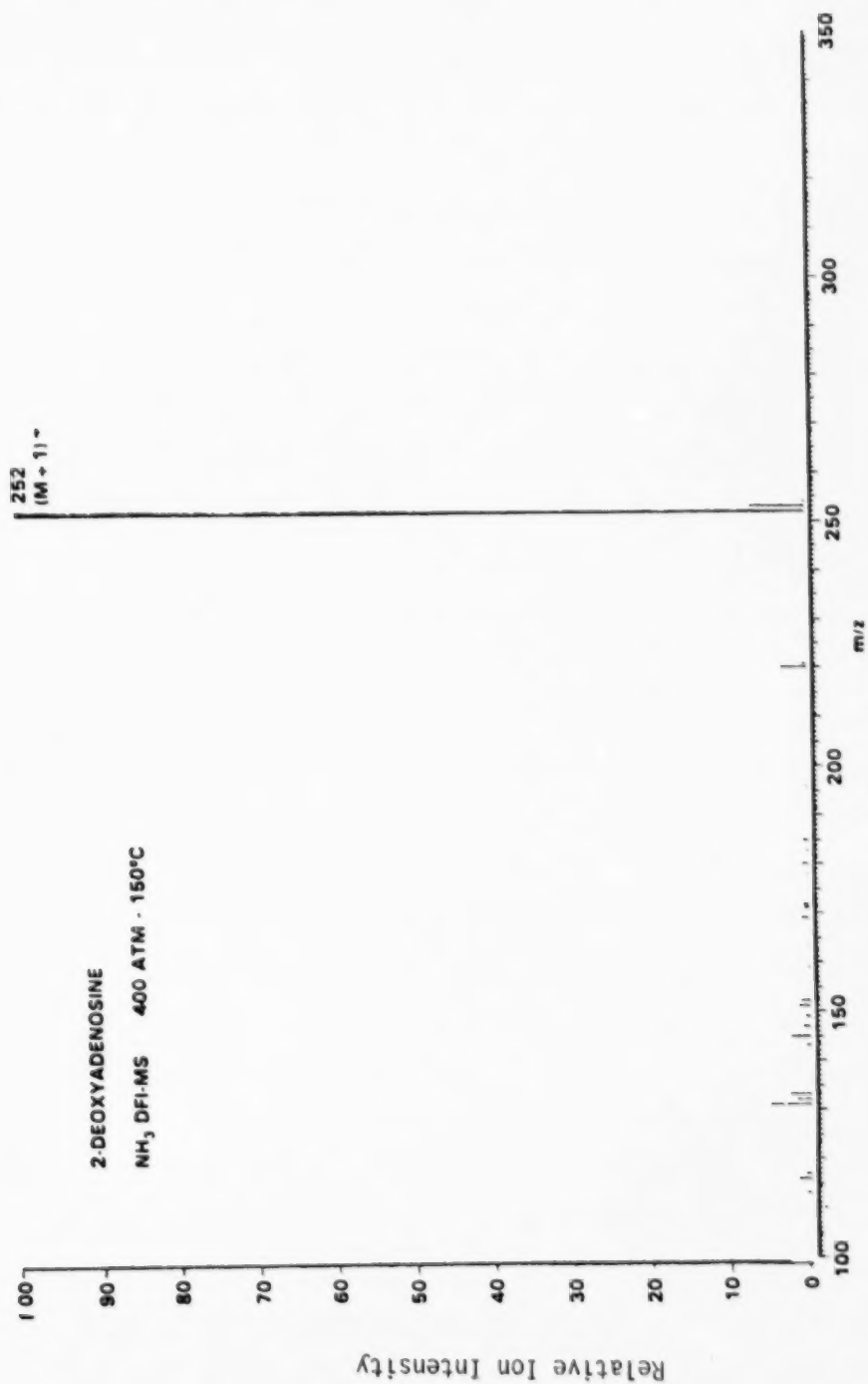


FIGURE 13. DFI-MS Spectrum of 2-deoxyadenosine Obtained with Supercritical Ammonia at 150°C and 400 atm.

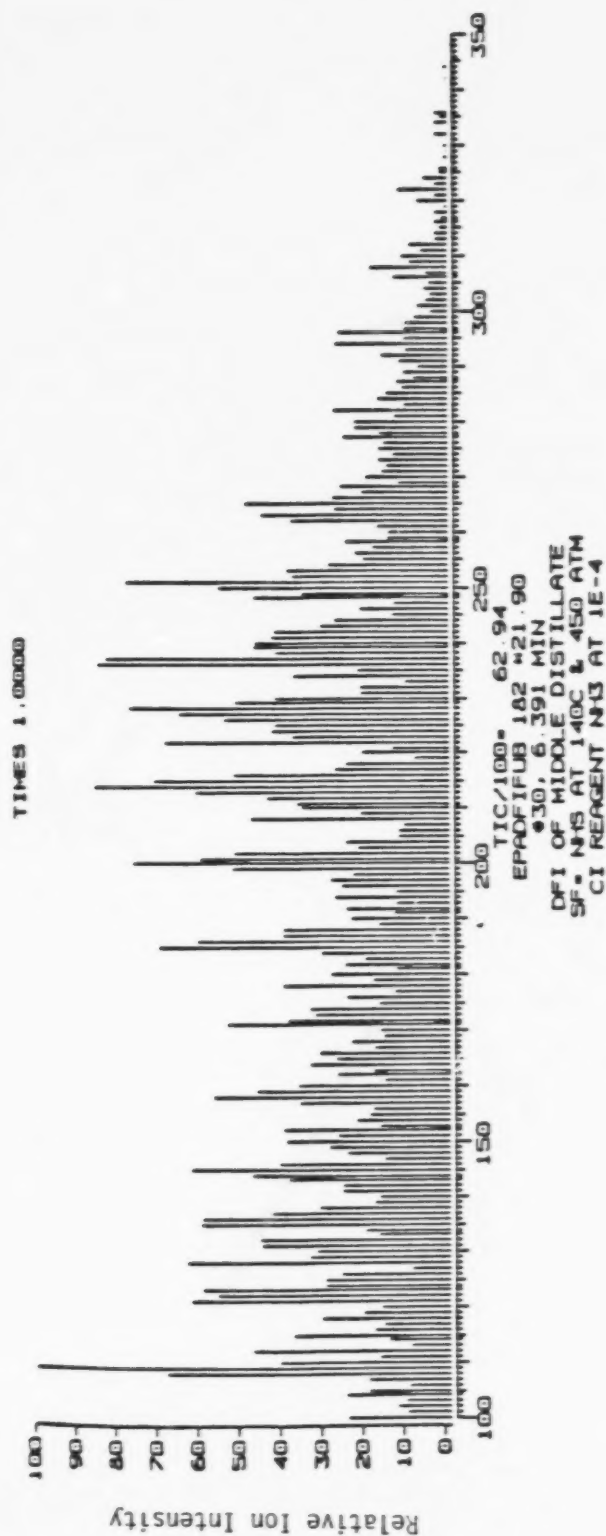


FIGURE 14. DFI-MS Spectra of the Middle Distillate Fuel Obtained with Supercritical Ammonia at 140°C and 450 atm.

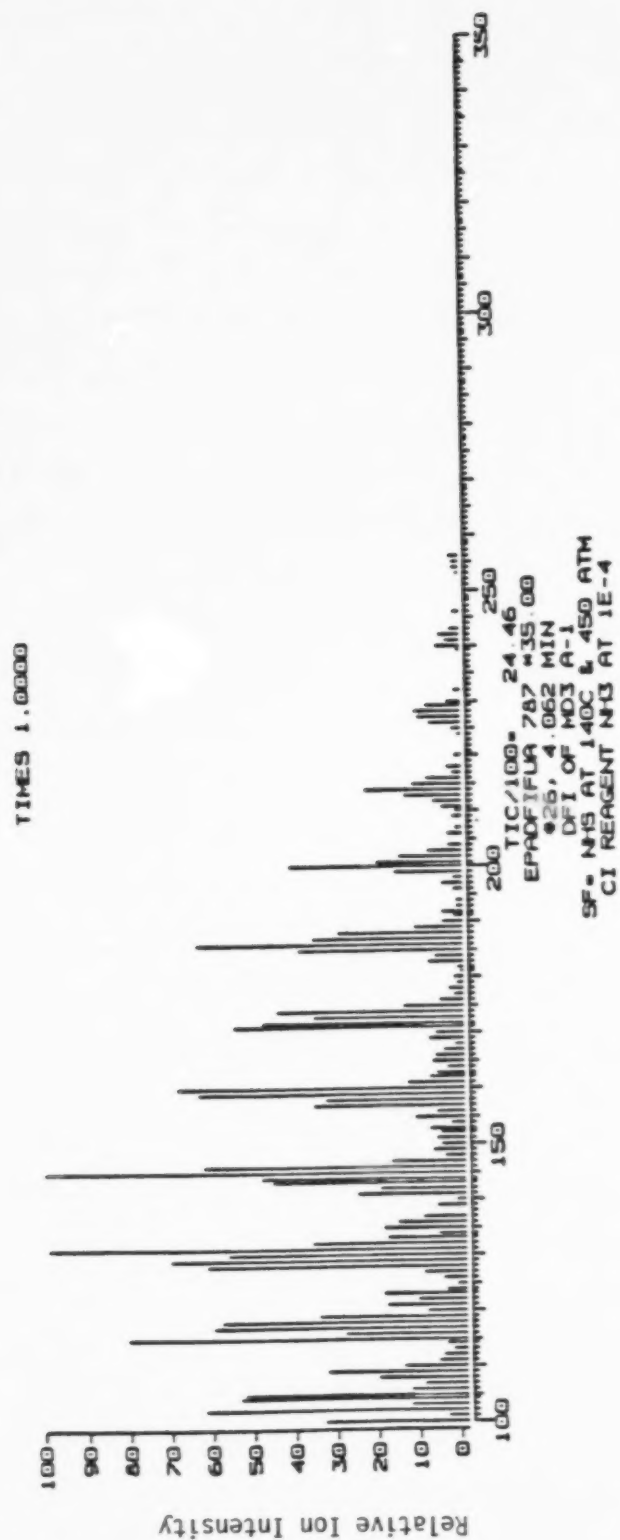


FIGURE 15. DFI-MS Spectra of the Aliphatic Hydrocarbon Fraction (A-1) of the Middle Distillate Fuel Obtained with Supercritical Ammonia at 140°C and 450 atm.

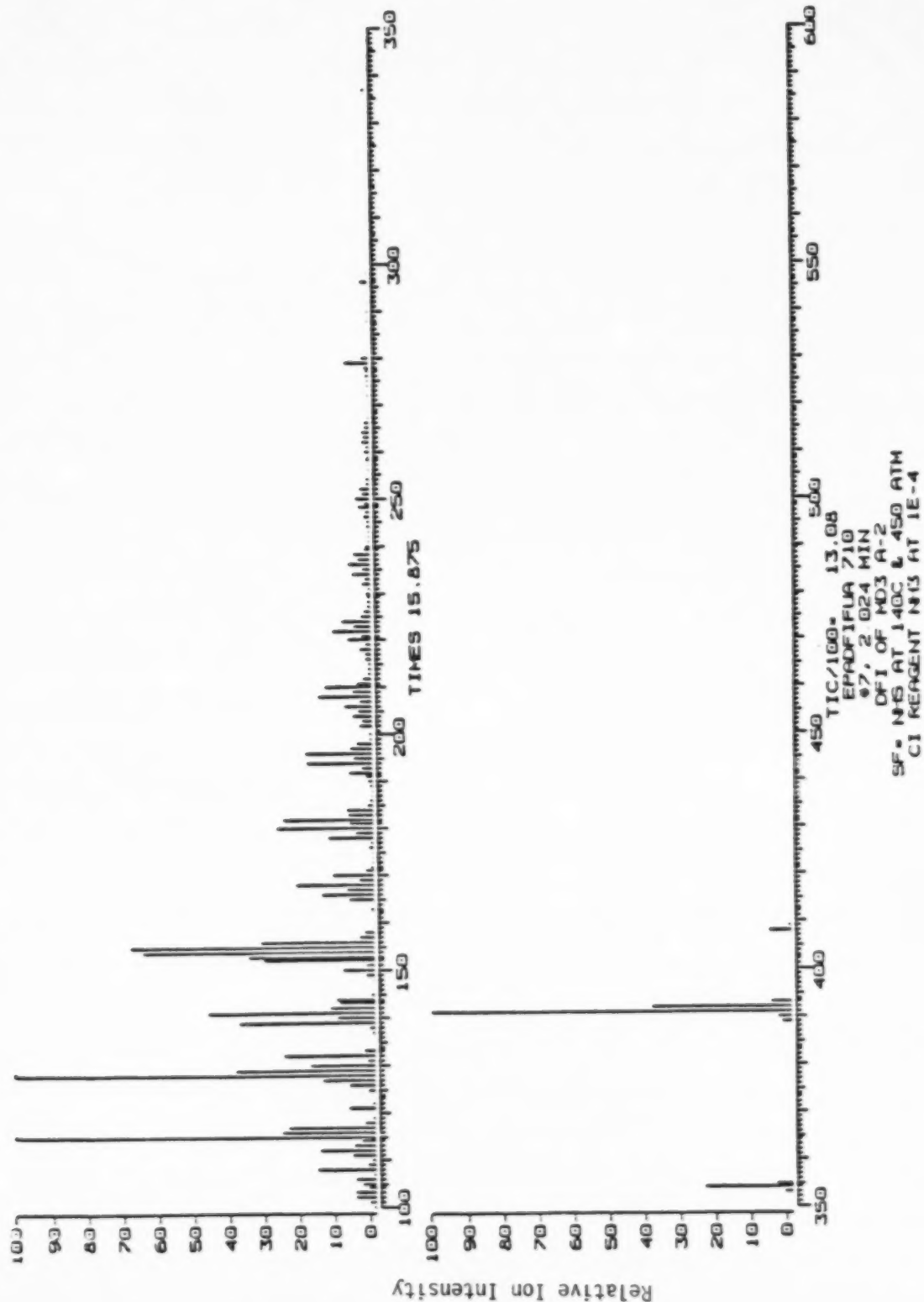


FIGURE 16. DFI-MS Spectra of the Polycyclic Aromatic Hydrocarbon Fraction (A-2) of the Middle Distillate Fuel Obtained with Supercritical Ammonia at 140°C and 450 atm.

TIMES 1.0000

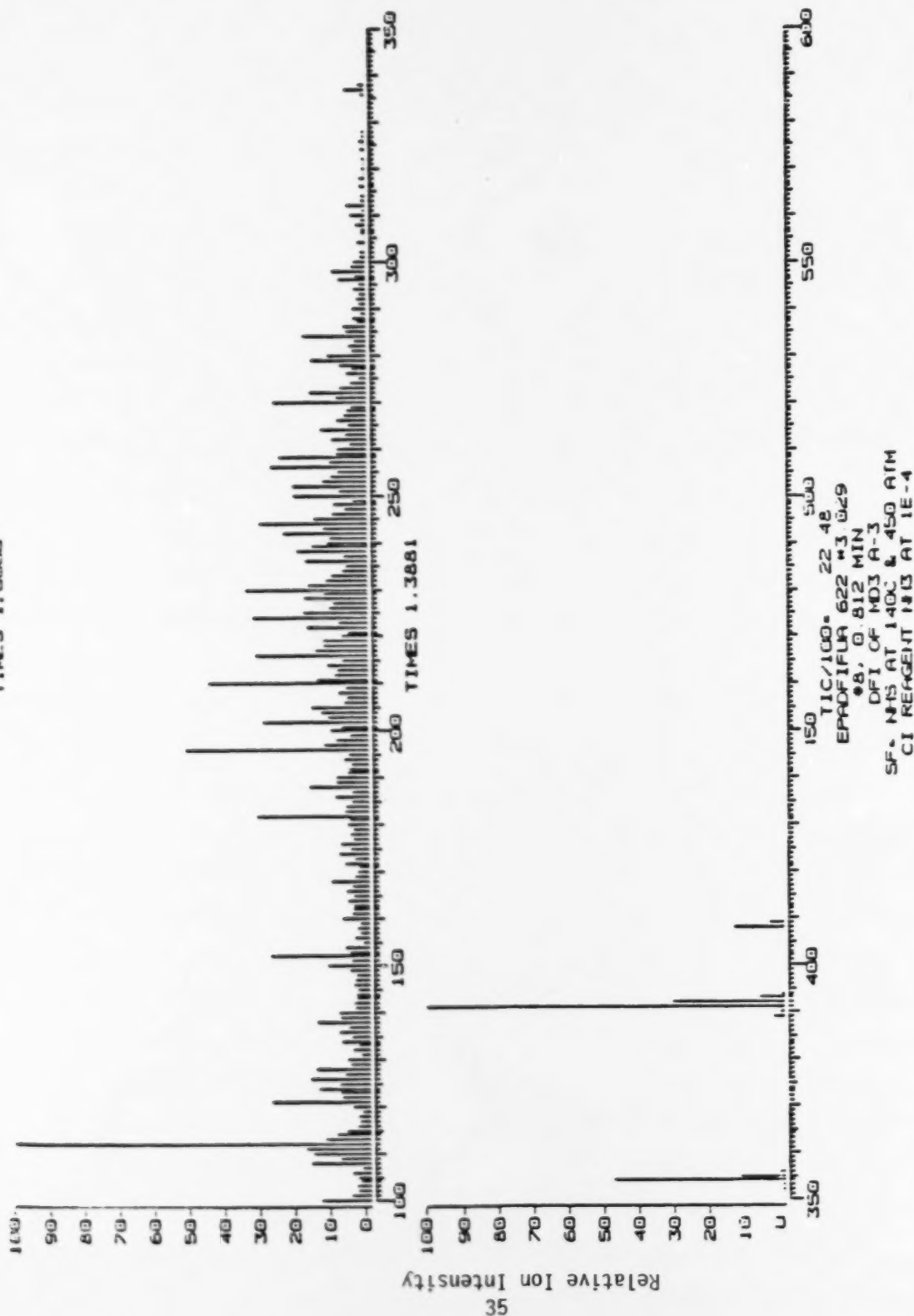


FIGURE 17. DFI-MS Spectra of the Nitrogen-Containing Polycyclic Aromatic Hydrocarbon Fraction (A-3) of the Middle Distillate Fuel Obtained with Supercritical Ammonia at 140°C and 450 atm.

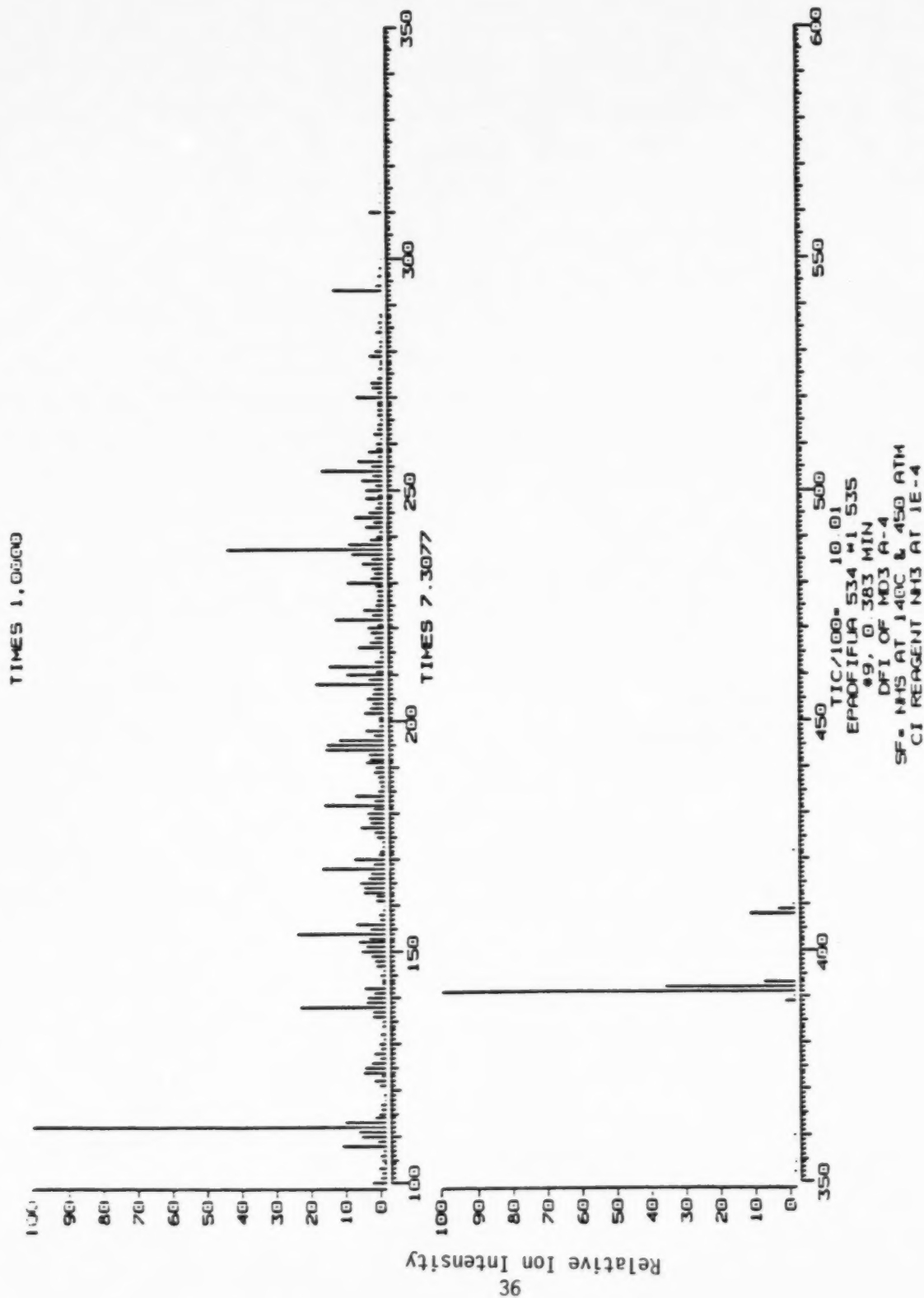


FIGURE 18. DFI-MS Spectra of the Hydroxy Polycyclic Aromatic Hydrocarbon Fraction (A-4) of the Middle Distillate Fuel Obtained with Supercritical Ammonia at 140°C and 450 atm.

around 349, 391, and 408 amu found in the spectra of the A-2 (Figure 16) and A-3 (Figure 17) fractions can be attributed to the solvent used for their injection. Blank analyses using benzene prior to injection of these samples had identical spectral features in these mass regions. Likewise, the 391 and 408 amu clusters in the spectra of the A-4 fraction (Figure 18) can be attributed to background material. The heaviest molecular weight material from the middle distillate fuel was found in the A-3 fraction (nitrogen heterocycles) ranging to nearly 350 amu. DFI-MS of the emission extract, however, gave evidence of material of molecular weight greater than 425 amu (see Figure 19). The large 391, 392 amu can again be attributed to background contaminants. The mass manifold obtained from this sample does not show any noticeable repeating features characteristic of homologous series, thus suggesting a wide variety of vastly different compounds are present.

The present results show that molecular weight distributions, as well as some qualitative inferences about structure can be obtained by DFI-MS of complex mixtures. Although experience with interpretation of the DFI-MS spectra is limited at this point, the DFI mass spectra are of similar complexity to those obtained by field ionization, and auxillary MS/MS methods are available for further characterization.

Supercritical Fluid Extraction

The emission particulates were extracted directly with supercritical ammonia and the effluent monitored continuously by mass spectrometry. Again, ammonia was used as the extracting fluid because of its excellent solvating properties, particularly for more polar materials. The extraction profile (total ion plot) obtained at 140°C as the ammonia pressure was ramped from 25 to 400 atm is shown in Figure 20. A large increase in the amount of material extracted is evident near the critical pressure. As the extraction progressed, the amount of material decreased, presumably

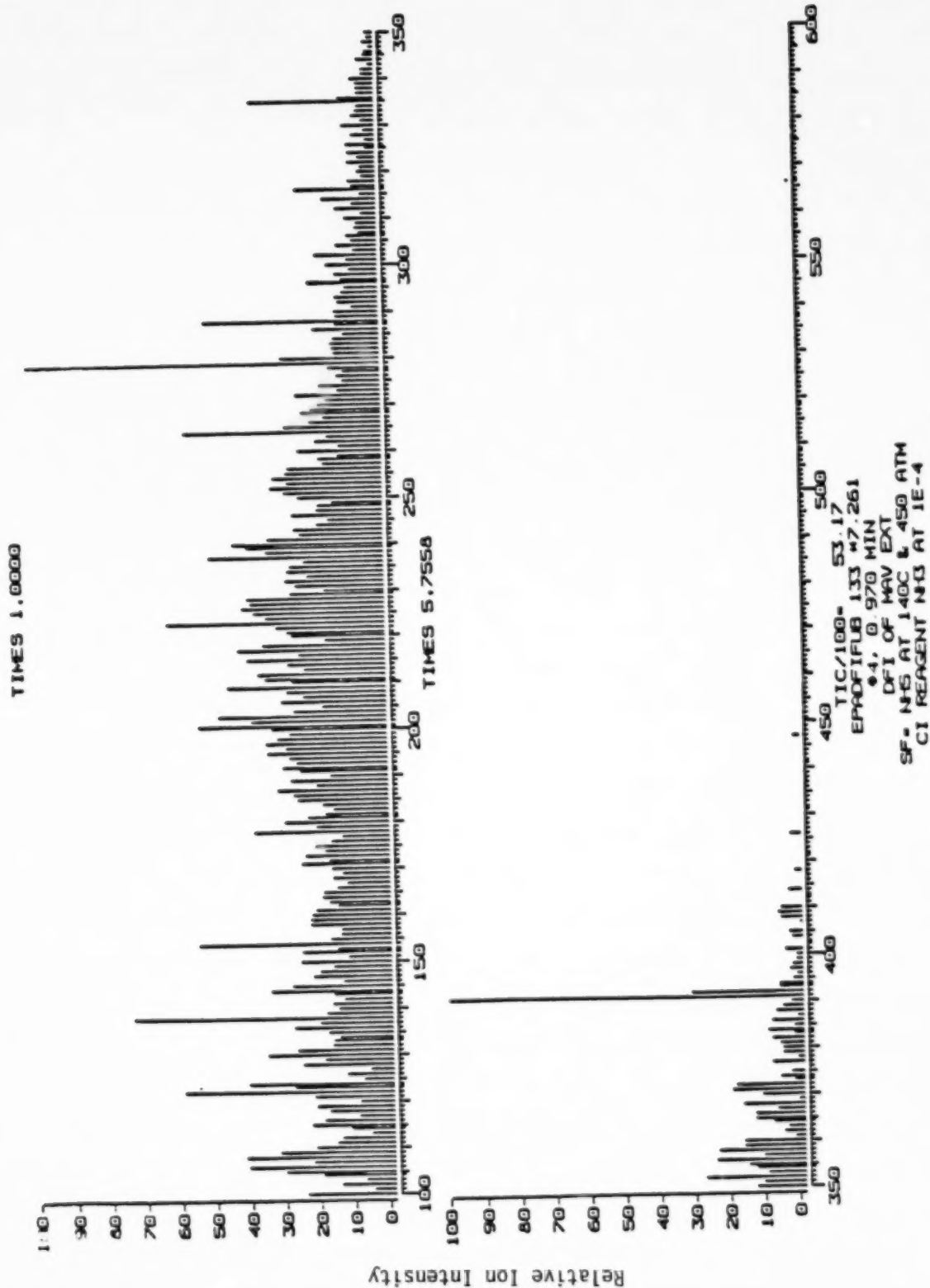


FIGURE 19. DFI-MS Spectra of the Emission Extract Obtained with Supercritical Ammonia at 140°C and 450 atm.

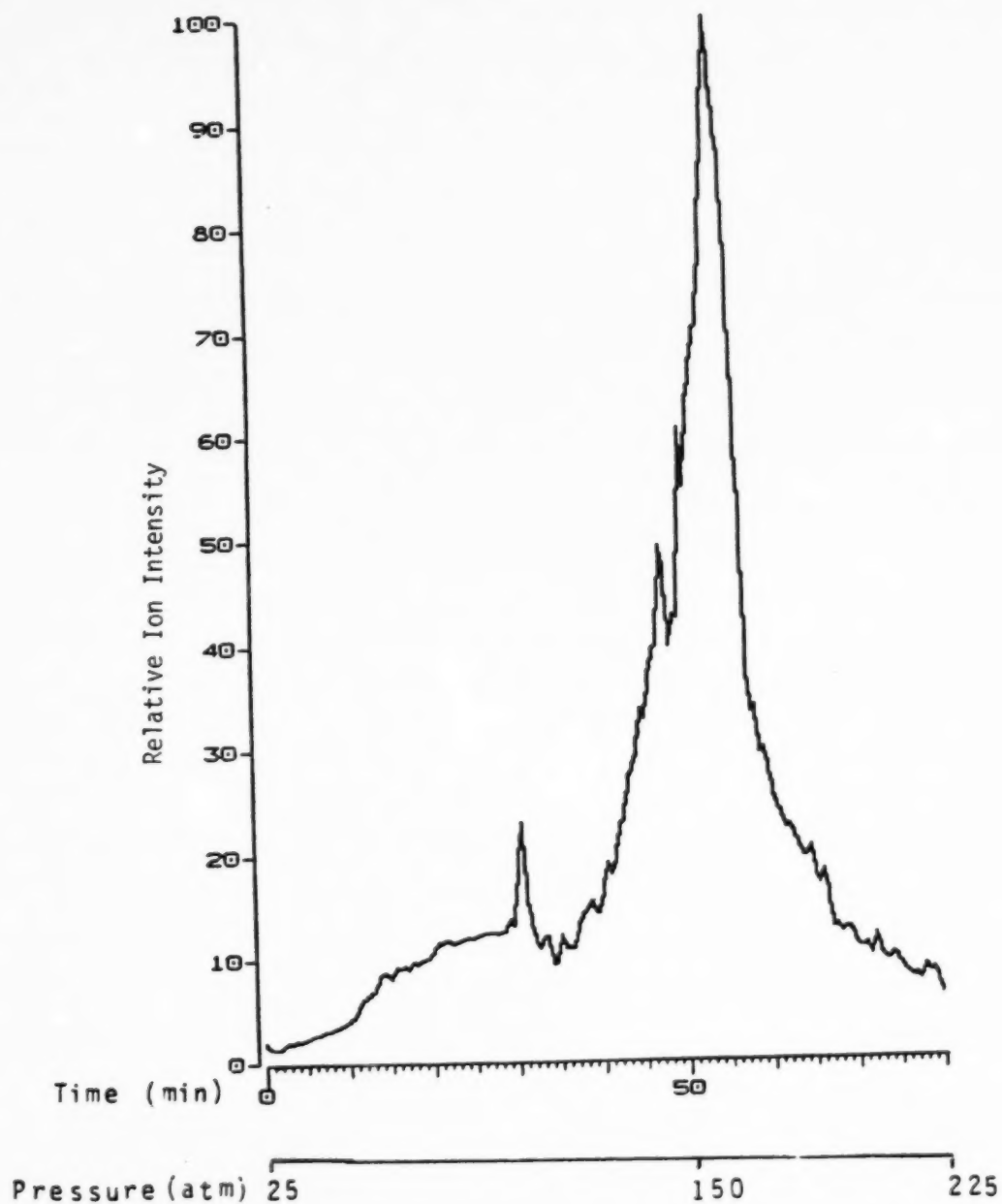


FIGURE 20. Total Ion Current Extraction Profile of the Emission Particulates Obtained with Supercritical Ammonia at 140°C as the Pressure was Ramped from 25 to 450 atm at 2.5 atm/min.

as it was exhausted from the extraction cell. After the pressure reached approximately 230 atm essentially no material remained. Spectra obtained at selected time intervals during the extraction are shown in Figures 21 to 25. At low pressure (36 atm), very little material above mass 250 amu was evident (Figure 21). As the pressure increased above 100 atm some material at higher masses was present (Figure 22). By 140 atm (Figures 23 and 24), material through the entire mass range was extracted. However by 200 atm (Figure 25) most of material had been extracted. The spectra obtained at a pressure of 192 atm from a blank run of the empty extraction cell are shown in Figure 26. A comparison of these spectra to those obtained from the sample clearly indicates that material up to molecular weight 1400 amu was extracted. The blank run also illustrates that no unusual NH_3 "clustering" effects were occurring at the high extraction pressures. However, the very regular peak clusters occurring approximately every 75 amu is somewhat suspicious (see Figures 23 and 24). Consequently, these data must be interpreted cautiously. Further investigations using MS/MS techniques or high resolution MS, and additional samples types are necessary to obtain conclusive information.

Quality Assurance/Quality Control

Since the primary objectives of this study were aimed at demonstrating the feasibility of several new supercritical fluid analytical techniques for organic analysis, a rigid and structured QA/QC plan was not applicable. The present work provides a preliminary application of these techniques and was not intended to provide a detailed or comprehensive sample characterization. However, standard analytical chemistry and good laboratory practices were followed.

All the data and information obtained during this program were qualitative in nature. The main efforts focused on characterizing the fractions under study rather than quantifying them. Consequently, no

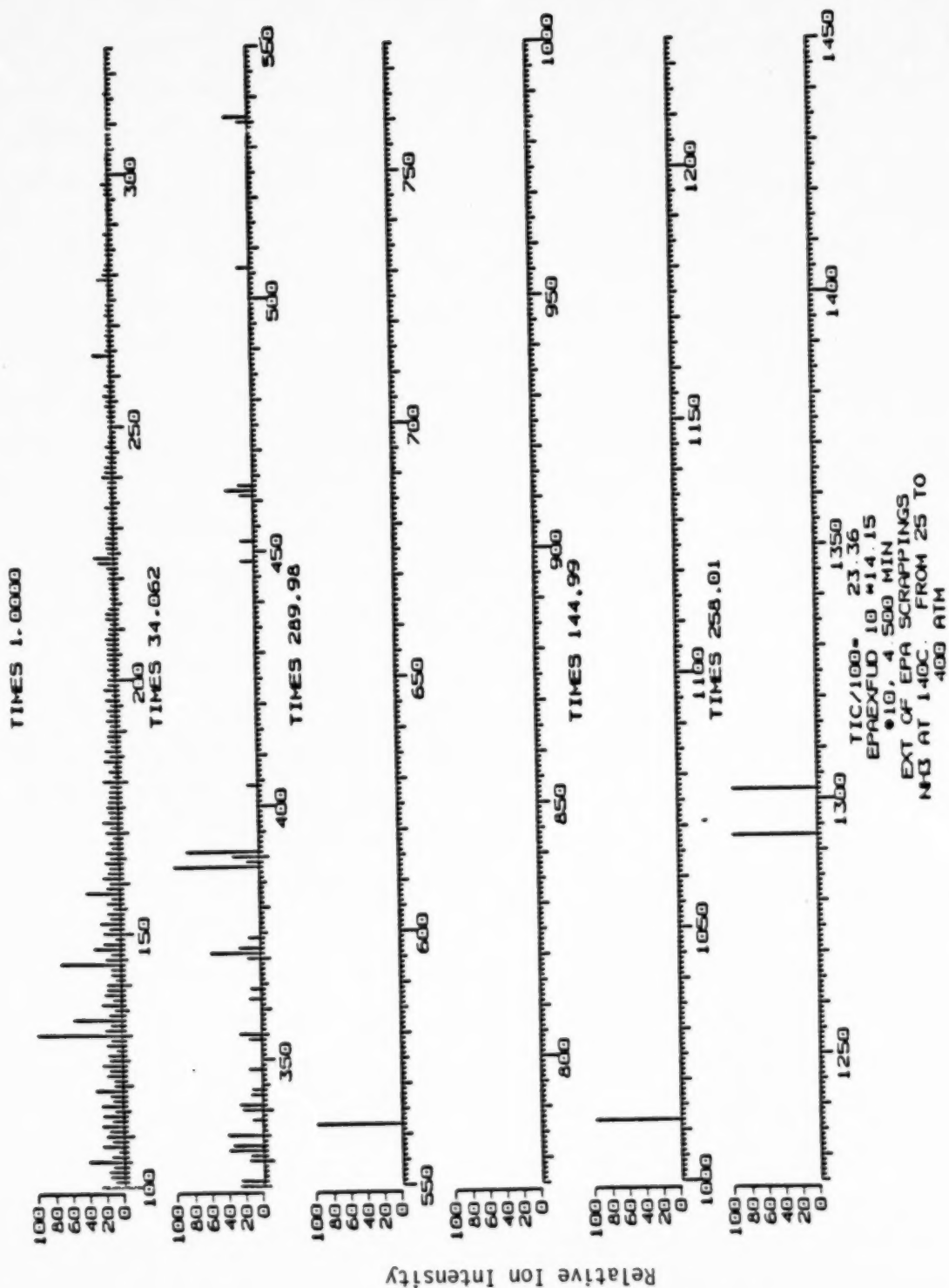


FIGURE 21. Mass Spectra Obtained from the Emission Particulate Extraction with Ammonia at 140°C and 36 atm.

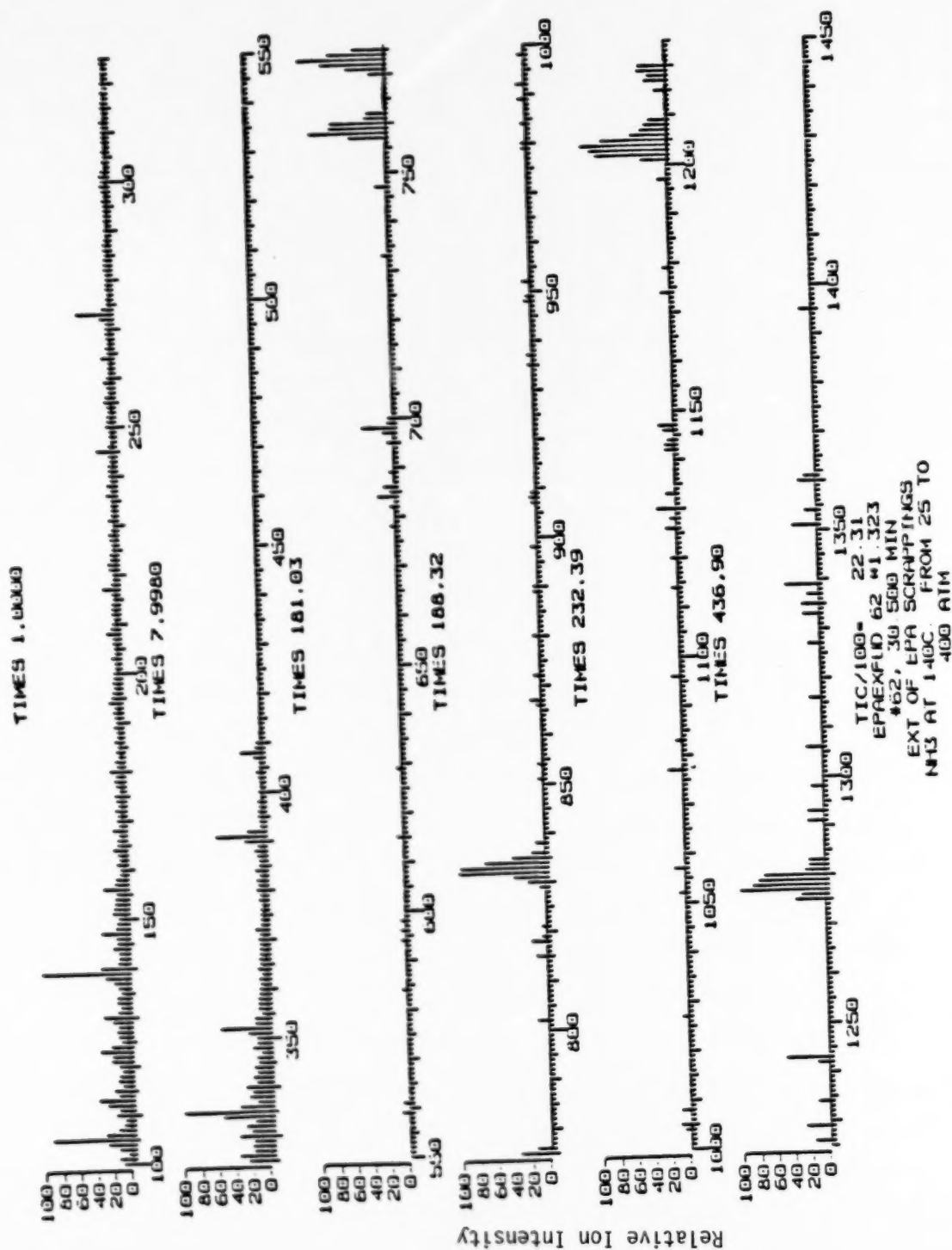


FIGURE 22. Mass spectra Obtained from the Emission Particulate Extraction with Ammonia at 140°C and 101 atm.

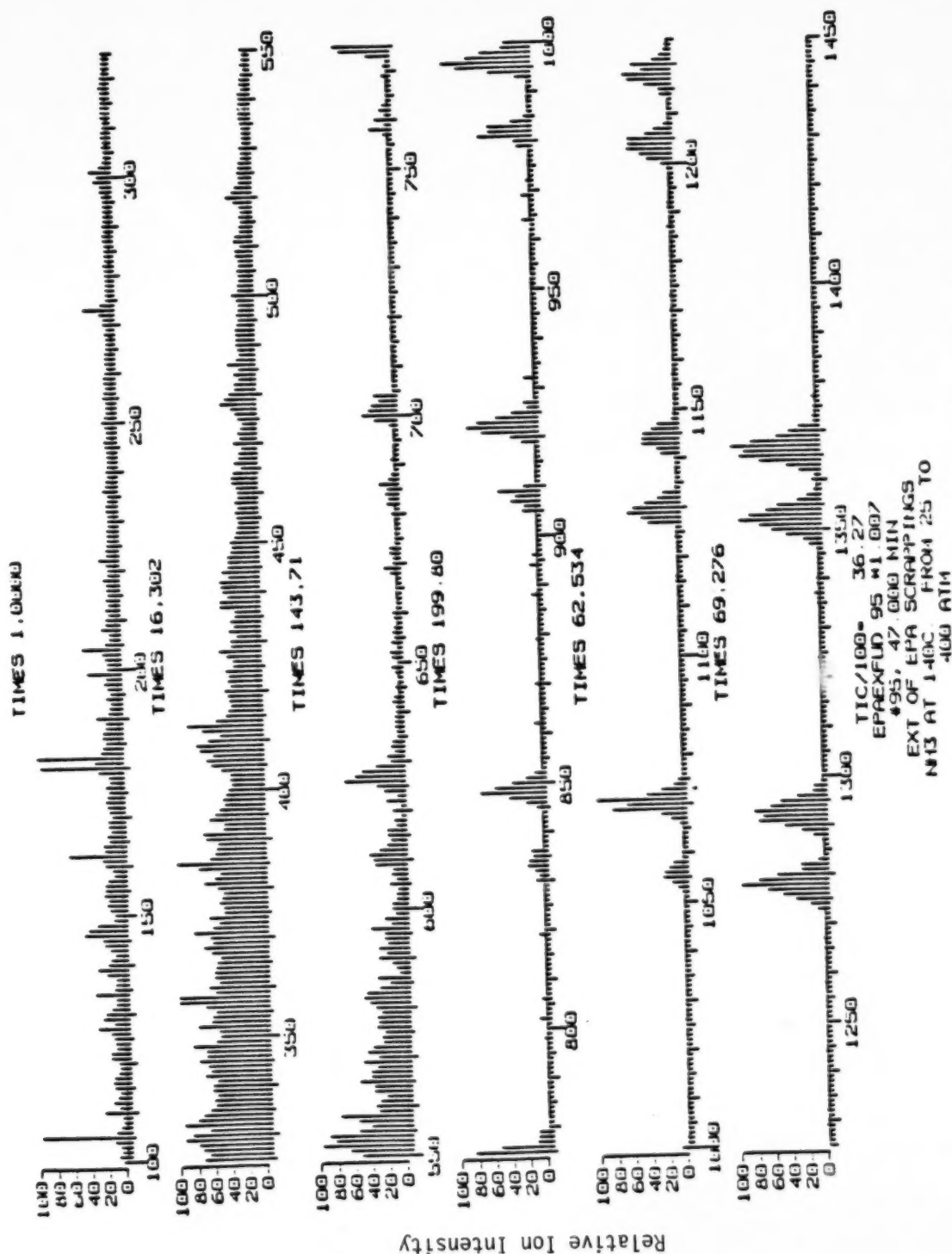


FIGURE 23. Mass Spectra Obtained from the Emission Particulate Extraction with Supercritical Ammonia at 140°C and 142 atm.

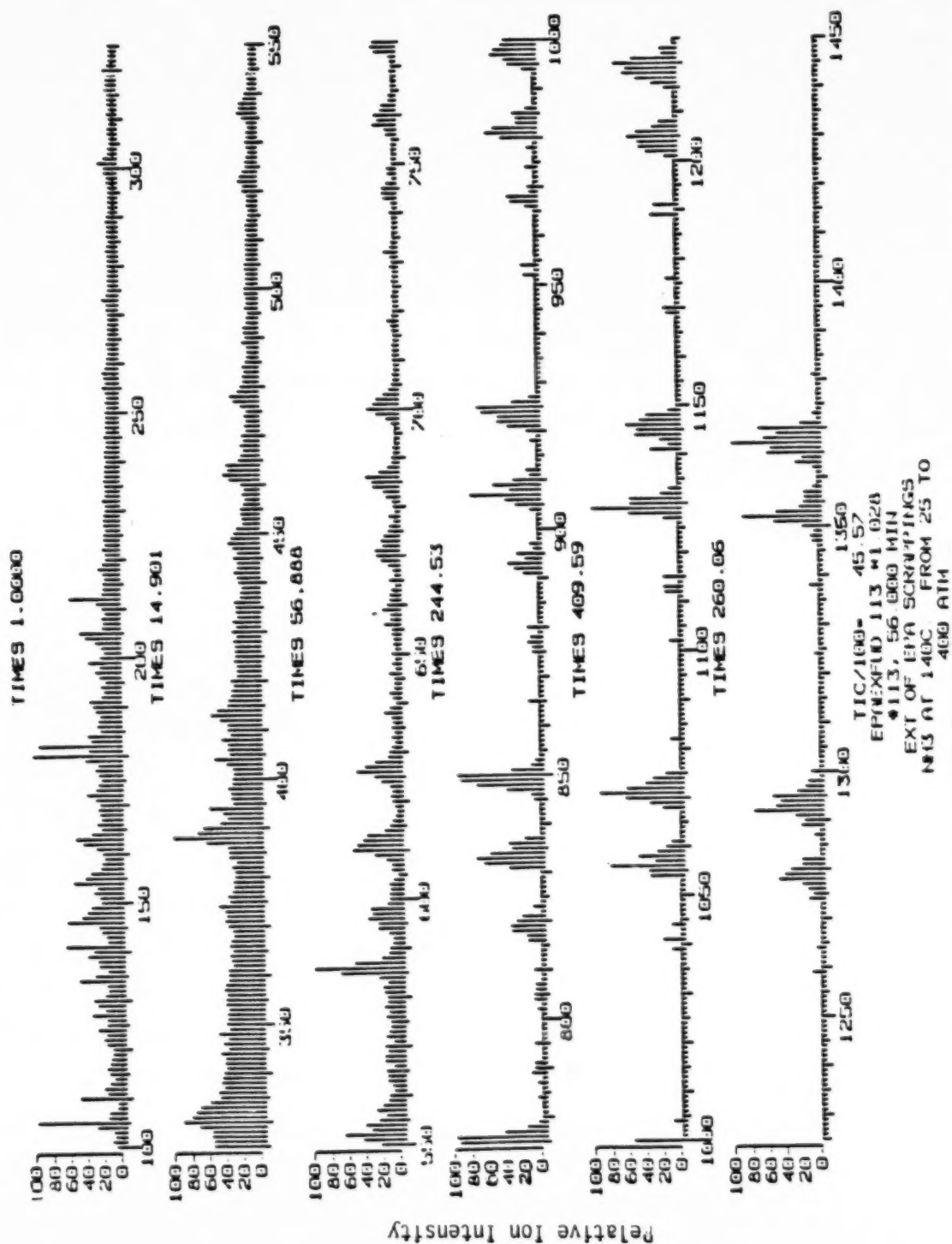


FIGURE 24. Mass Spectra Obtained from the Emission Particulate Extraction with Supercritical Ammonia at 140°C and 165 atm.

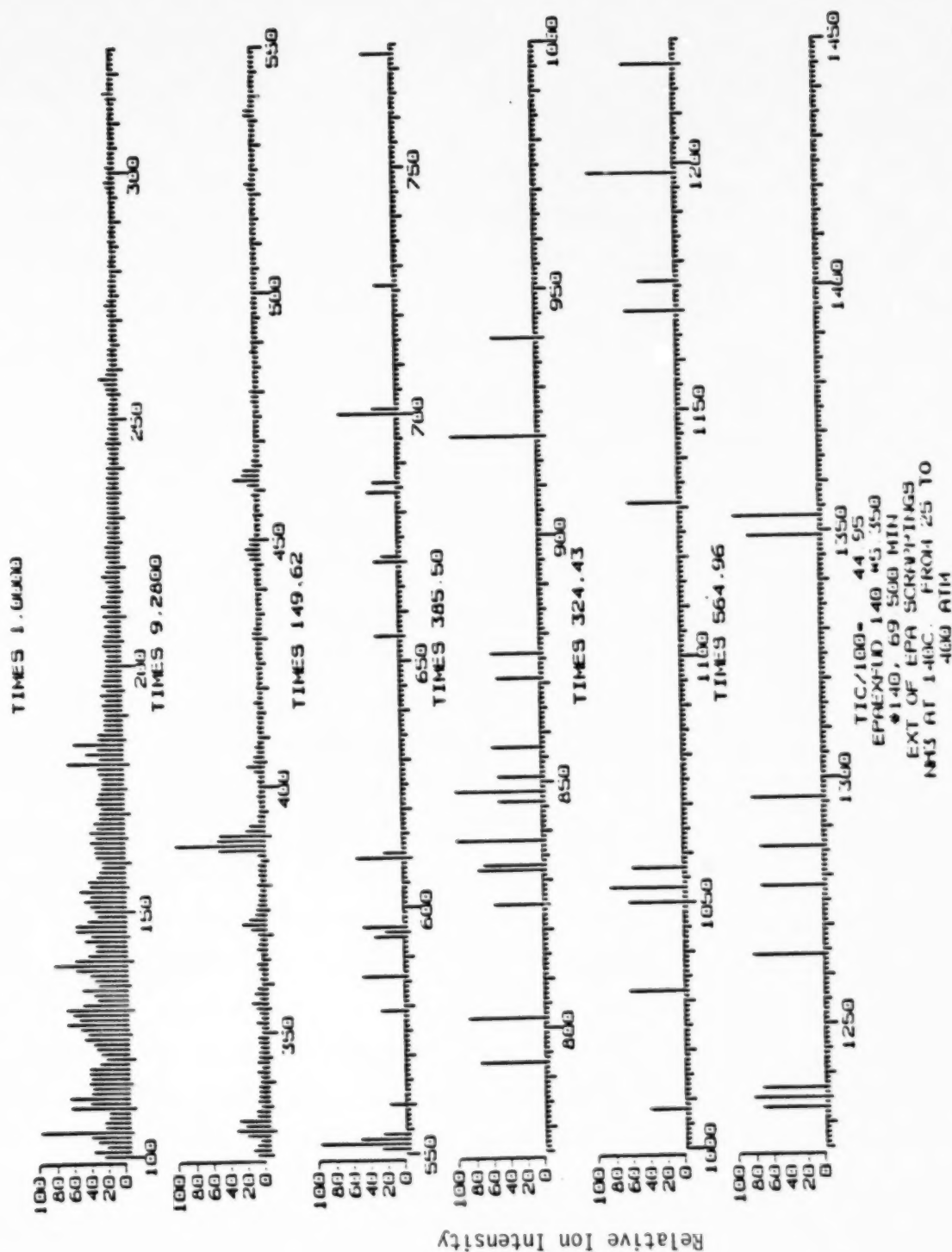


FIGURE 25. Mass Spectra Obtained from the Emission Particulate Extraction with Supercritical Ammonia at 140°C and 200 atm.

TIME 1.0000

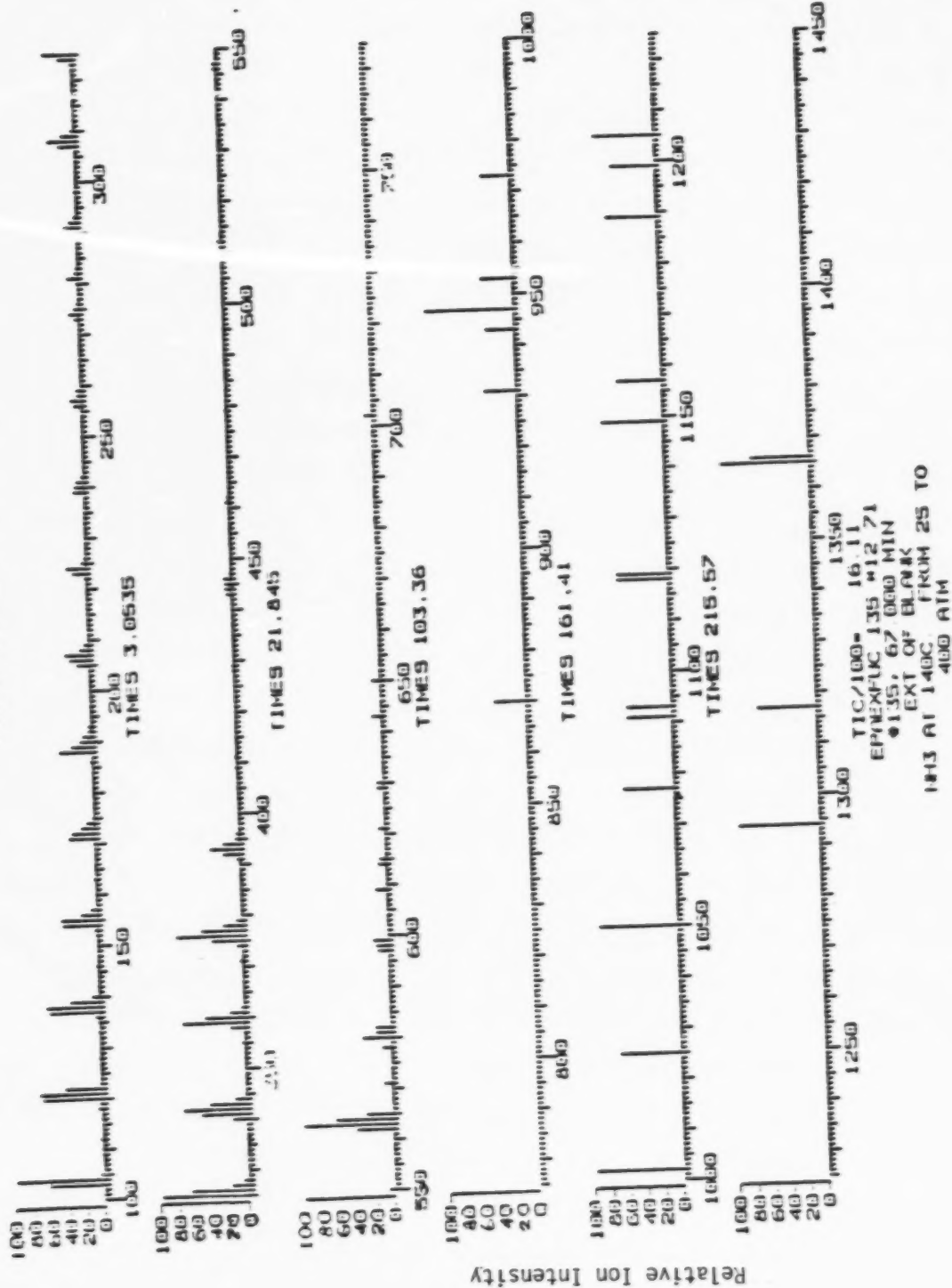


FIGURE 26. Mass Spectra Obtained from a "Blank" Analysis of the Empty with Supercritical Ammonia at 140°C and 200 atm.

calibration curves were prepared nor accuracy and precision studies performed.

Since the identification of the compounds in the samples from this study was for methods research only, the identification should be regarded as tentative and no quality assurance should be assigned to the identifications.

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